ANNUAL REPORT 2004















Entomology Unit

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1. Introduction



The very successful implementation of SIT programmes for control of medfly, Ceratitis capitata, *and the increasing trend to use sterile insects for pest suppression, as opposed to eradication, has led to a mounting interest in commercialization of the technology. This is a very welcome development and should greatly help in the sustainability of the technology. There are now several fledgling companies established.*

A very large colony of the olive fly, Bactrocera oleae *has now been established in the Unit and material from this colony is now being supplied to many colleagues in Member States. In addition, a new field strain from Spain has been successfully hybridized with the very old established colony. A new larval diet component has been successfully evaluated to replace the very expensive cellulose and standard reference diet has been established. Activities in 2005 will focus on field cage evaluations of competitiveness and the replacment of the current a the current protein source with a cheaper alternative.* Anastrepha fraterculus, *attention will be focused on improvements to the oviposition cage. In relation to a Coordinated Research Project on codling moth,* Cydia pomonella, *SIT several field samples of diapausing larvae of this species from different parts of the world have been received in Seibersdorf. In 2005, mating compatibility tests will be carried out with these populations by consultants from Argentina.*

The evaluation of containerized rearing for tsetse was successfully completed in 2004 using Glossina fuscipes. *As mentioned in ANNUAL REPORT 2003, the plan was to install a version of TPU 3.2 in the container, this is still pending, however, two rearing rooms in the tsetse rearing area have been modified and a complete TPU 3.2 has now been installed. This will provide an opportunity to train fellows in the use of the technology.*

The mosquito rearing laboratory was fully functional and two colonies of Anopheles arabiensis *are being reared. The first is a well established laboratory colony, KGB, and the second a colony successfully established from the project area in Dongola, Sudan. Mark Benedict returned to the Unit in 2004 and will continue to divide his time between CDC and the mosquito group for the next two years. Three consultants joined the group in 2004, Mr. H. Bossin to develop improved rearing protocols and transformation technology, Ms. Michelle Helinski to develop radiation sterilization protocols and Ms. Genevieve Labbe to support the work on transformation.*

Early in 2005, Mr Gratian Mutika returned to Zimbabwe at the end of his contract and Mr. H. Mohammed joined the tsetse group in January 2004 and is responsible for organizing the blood diet for the tsetse colonies.

1.1 Sexing Tsetse Fly Pupae

The ability to automate sexing of tsetse flies would greatly increase the efficiency and flexibility of operational SIT programmes. Currently this is done by exploiting the difference in developmental times between females and males in the Self Stocking Production Cage system (SSPC) (ANNUAL REPORT 1999). However, this system has some disadvantages and during 2004 it was investigated as to whether infra-red spectroscopy could be used to differentiate male a female *Glossina pallidipes* pupae. During a consultancy in the Unit, Dr. Floyd Dowell (ARS, Kansas) was able to demonstrate the effectiveness of this technique for tsetse and to identify the sex of pupae five days before emergence with considerable accuracy. The screening of individual pupae is fast enough to be used in an operational programme and further work will be carried out in 2005 to fully validate this technology for *G. pallidipes* and assess its usefulness for other tsetse species.

1.2 Radiation Environment for Medfly Pupae

Current operational practice for irradiation of medfly pupae involves packing pupae two days before emergence in plastic bags, allowing anoxic conditions to develop in the bag due to pupal metabolism and then carrying out radiation in an atmosphere of reduced oxygen tension. Although this practice is standard, the level of anoxia induced during different time periods and at different holding temperatures remains unknown and the procedure is not standard in terms of time and temperature. Dr. David Nestel (Volcani Institute, Israel) spent some time in the Unit to try to actually measure the environment within the plastic bag at different time following sealing and at different temperatures. The goal is to develop a more precise set of guidelines to ensure equivalent treatment of pupae at the different rearing facilities and to quantify the anoxic conditions during radiation.

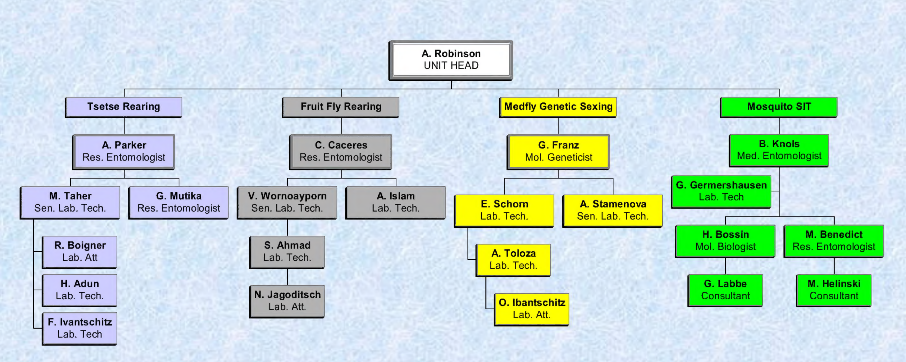
1.3 Tsetse Diet

The blood used to feed tsetse is the single dietary component required but it is a biological product that brings with it certain logistic problems in terms of availability, collection and biological contaminants e.g. pathogenic bacteria. Currently blood collected in abattoirs is sterilized by radiation before use, however in certain Member States this is not possible as relatively large radiation sources are required. There are recent developments in the medical field in relation to the safety of blood used in transfusions that may be of relevance to sterilization of tsetse blood diet. Contacts have been initiated with IATRO, a small company in the UK, who are involved with blood sterilization using UV radiation. A contract has been issued to the company to evaluate this technology for sterilization of tsetse blood diet and to loan equipment to the Unit for a six-month period. In related work analysis is progressing on the isolation of the so-called “yellow factor” from bovine blood. Previous work has shown that this factor is important for tsetse nutrition and it could be used an additive if freeze-dried blood was required to be used. This work is carried out by a cost-free intern who is registered for a PhD at the University of Vienna.

1.4 Bactrocera Genetic Sexing Strains

Colleagues at USDA/ARS in Hawaii have developed potential genetic sexing strains based on white pupal colour mutations for *Bactrocera cucurbitae* and *B. dorsalis*. These have now been transferred to the quarantine facility in the Unit where detailed quality control and stability evaluations will be carried out.

ENTOMOLOGY UNIT ORGANIZATIONAL CHART



2. Quality Control and Rearing Studies in Tsetse

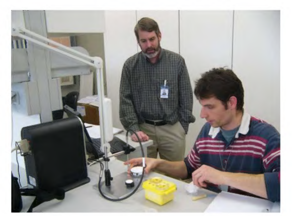
*The evaluation of tsetse rearing container was completed, using **G. fuscipes**, following the establishment of suitable environmental conditions. The evaluation showed that containerized rearing of tsetse is feasible and that production levels are equivalent to those in a conventional insectary. The evaluation, as completed was not fully independent as certain activities had to be carried out in the normal insectary, e.g. cage stocking, tray washing etc. However, as these are less sensitive to environmental conditions it is not expected that they will provide a hurdle for complete self-contained rearing. The flexibility provided by a container based rearing system now needs to be fully evaluated in terms of facility development in affected Member States together with a full economic analysis for the different scenarios available.*





A preliminary evaluation of the TPU 3.1 rearing system was reported in the ANNUAL REPORT 2002. This prototype was very limited in extent and significant engineering improvements have been made since then and the version TPU3.2 will be installed in Ethiopia in the near future. In order to provide support and fellowship training on this new rearing system a complete unit has been purchased and been installed in the tsetse rearing area. The unit will be able to hold a colony of 70,000 breeding females. In 2005 a full evaluation of the system will be carried out covering both the productivity of the system and the ease of operation.

Significant progress has been made in the development of an automated system for the sexing of tsetse pupae. If successful this will have a major impact both on the efficiency of operational SIT progammes but also on their flexibility. Preliminary work in 2003 showed some promise and this was expanded in 2004 with very encouraging results. The system relies on the use of infra-red spectroscopy which has been developed to identify cereal grains that contain an insect pest. Using this equipment it was possible to get very good discrimination between male and female pupae when they were analysed 5 days before adult emergence.



If this can be confirmed it would enable stocking of cages to be accurately and simply done and also irradiated pupae to be shipped to release sites as is currently done with most SIT programmes for other species.

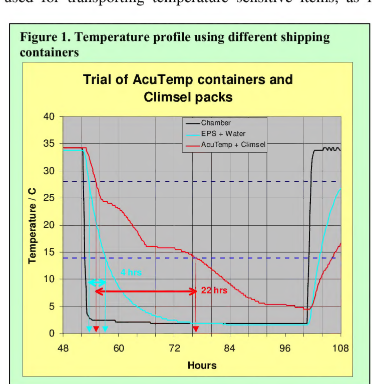
2.1 Packaging for Pupal Shipment

Following the work reported in the 2003 ANNUAL REPORT, work continued to find yet better packaging materials for shipping pupae. Two commercial products were identified and tested, AcuTemp shipping boxes and Climsel phase change materials.

The first improvement was to the box used for shipment. Previously pupae were shipped in expanded polystyrene boxes, 220x190x125mm external, with a 17mm wall and external cardboard shipping carton. When measured this box gained energy at the rate of 6.8 W/°C. AcuTemp shipping boxes are constructed from open cell rigid foam encapsulated in metal foil, which is then evacuated to about 5kPa. The foam provides a degree of insulation, but the evacuation increases the insulation analogous to a Dewer flask. The boxes used for pupal shipment are 203x150x135mm external, with a 12.7mm wall thickness, with inner and outer cardboard liner and shipping carton. R values of 25 or greater are claimed, compared to 4 for expanded polystyrene, but in tests in a temperature control chamber the heat gain was about 2.8 W/°C, considerably less than the R values would suggest. This may be partly due to the relatively poor fit of the lid on the AcuTemp box.

The second improvement was in control of the temperature inside the box. As reported in ANNUAL REPORT 2003, simply increasing the thermal bulk of the contents using sachets of water significantly stabilized the temperature. Ice is often used for transporting temperature sensitive items, as it has a large latent heat of fusion. Tsetse pupae cannot tolerate temperatures below 10°C so ice is not appropriate, but other substances with a phase change around 20°C could be used for this purpose. Glycerine was fist tested as it has a melting point close to 20°C, but it super-cools rather than crystallizing. Two groups of compounds have phase changes in the appropriate range, paraffin waxes and salt hydrates, both of which are used as heat storage compounds for building management and such like. The materials chosen for testing were salt hydrates, which are available in a number of formulations with phase transition temperatures from 4–48°C. Two formulations were obtained from the company Climator, with phase change temperatures of 18 and 24°C (Climsel C18 and C24). For the salt hydrates the phase change is not as sharply defined as the melting of water, but occurs over a span of 2–3 degrees. **Figure 1** shows the results of a trial to compare a standard expanded polystyrene box with 500g water with an AcuTemp box

Figure 1. Temperature profile using different shipping containers



with 250g C18 and 250g C24 Climsel. After stabilization for 48h at 34°C the chamber temperature was dropped rapidly to 3°C and the temperature inside each box recorded every 10min. Taking 28°C as the upper limit for transporting tsetse pupae and 14°C as the lower limit, the time taken for the expanded polystyrene/water combination to fall from 28–14°C was about 4 hours, whereas the AcuTemp/Climsel combination takes about 22h. The phase changes occurring in the Climsel can be clearly seen as a plateau in the temperature profile at 24 and 16°C. Simulation of a real transport temperature profile shows that this combination of packaging box and phase change material can maintain the temperature within an acceptable range over an extended period. This system has now been implemented for shipments to Tanzania but as yet no feedback has been received as to whether or not the survival of pupae has been improved.

2.2 Colony Status

The colonies have been held at an almost constant level throughout 2004, after the initial reduction in January due to staff reductions. Despite these reductions slightly more than 500,000 pupae were shipped during the year to insectaries in Africa and researchers throughout the world (**Table 1**). In December the *G. pallidipes* colony was reduced from approximately 25,000 to approximately 15,000 in preparation for the move necessitated by the building work required for the installation of TPU3.2. A detailed list of the shipments can be found Section 6.4.

Table 1. Status of tsetse colonies and shipment totals

Species	Origin of strain	Colony 01/01/04	Colony 31/21/04	Shipments 2004
G. brevipalpis	Kenya, 1987	3,842	4,293	55,235
G. f. fuscipes	CAR, 1986	6,818	5,647	92,880
G. m. morsitans	Zimbabwe	8,996	5,916	77,000
G. m. centralis	Tanzania	9,580	6,037	28,028
G. pallidipes	Uganda, 1975	34,010	17,234	195,174
G. p. palpalis	Nigeria, 1981	4,982	5,100	53,300
G. swynnertoni	Tanzania, 1989	121	841	0
Totals		68,349	45,068	501,617

2.3 Tsetse Rearing in Slovakia

The Institute of Zoology of the Slovak Academy of Sciences (IZ-SAS), under a contract with the IAEA initiated in December 2002, has established a tsetse rearing facility to support the work of the Entomology Unit. Throughout this time the Entomology Unit has been providing technical advice to IZ-SAS, including monthly visits to the rearing facility.

IZ-SAS was selected for this contract because of its lower production costs and the ease of access from Seibersdorf to Bratislava, which permits regular contact. The idea of the contract arose because of the planned reduction in the rearing at Seibersdorf, so as to maintain the Agency's ability to supply to rearing facilities in Africa adequate quantities of tsetse pupae in a timely manner. Conversion of a former animal house area started in mid 2003 and was completed later the same year. Three species of tsetse have been provided from the Seibersdorf to establish the colonies. Initially problems were encountered and the flies were not thriving, but a concerted effort, coupled with a visit to the insectary by experienced technicians from Seibersdorf, resulted in a number of changes of procedure and succeeded in resolving

the problems. By late 2004 the colony stood at about 80,000, and reached the target colony size of 100,000 early in 2005.

2.4 Blood Treatment

Blood is the major consumable required for tsetse rearing. In order to obtain sufficient supply, blood must be collected from the abattoir at slaughter and processed for tsetse feeding. Collection at the abattoir cannot be made sterile, and the defibrination process often increases the level of contamination. Standard processing involves mechanical defibrination and freezing for storage, and treatment with 1 kGy gamma radiation to reduce bacterial contamination.

The reliance on gamma irradiation to control bacterial contamination severely restricts the opportunity for blood collection and processing in Africa, as large gamma irradiators are uncommon. We are therefore investigating alternative methods of blood processing, both to minimise the bacterial contamination at collection, and to reduce the contamination after collection. The main change in collection procedure being investigated is the use of citrate anticoagulant as an alternative to defibrination (ANNUAL REPORT 2003) and this work is continuing.

Control of bacteria can be achieved by irradiation, heat treatment and chemical treatment. Apart from gamma irradiation, we will also investigate UV irradiation during 2005. Heat treatment, specifically pasteurization, is a standard method of bacterial control employed extensively in the food industry. Initial work indicated the possibility of HTST pasteurization at about 70°C for less than 10 seconds. Under a research contract times and temperatures in this range are being tested, but it seems that the practical problems in controlling the conditions will probably make this impractical. Work on this will continue in parallel with the UV treatment.

The final possibility is chemical treatment. Bacteria can be controlled by use of antibiotics, but antibiotics cannot be used in the feeding of tsetse flies as they also kill the obligate symbiotic bacteria that are essential for normal reproduction in tsetse. The phenothiazine dyes, which include such familiar compounds as gentian violet, have long been know to kill bacteria and protozoa, and some have been routinely used in human blood transfusion to prevent transmission of Chagas disease. The dyes bind specifically to DNA and are used as microscopic dyes to contrast chromosomes. They cause damage to DNA by generating free radicals, the production of which is enhanced by irradiation with red light of various wavelengths around 600nm, the wavelength depending on the specific dye.

We decided to test four of these dyes mentioned in the context of human blood transfusion, methylene blue (MB), dimethyl methylene blue (DMMB), neutral red (NR) and azure. The first step was to test their acute toxicity for tsetse. For this the standard 25-day feeding test was used, which measures survival and production of pupae in the first ovarian cycle. **Figure 2** shows the average of four replicates for each dose of each dye, the doses being selected to span the dose used in blood transfusions, 1 or 10µM depending on the dye. The dye treated blood was fed to the flies without specific red light irradiation. A value of QFC25 > 1.1 is considered acceptable for normal colony feeding.

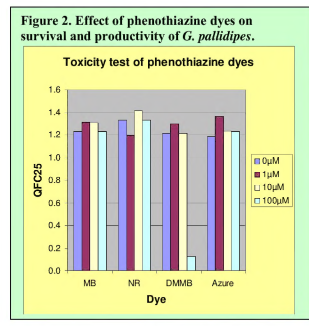


Figure 2. Effect of phenothiazine dyes on survival and productivity of *G. pallidipes*.

None of the dyes shows acute toxicity at the active concentrations of 1 and 10µM, and only DMMB is toxic at 100µM. DMMB is a more active analogue of MB, and is typically used at only 1µM, so the acute toxicity at 100µM does not cause a problem. Preliminary tests of 1 and 10µM concentrations of MB, DMMB and NR indicate that they achieve a 3 log or greater reduction in *Bacillus* and *Staphlococcus* in blood stored at 4°C for 24h. This work will continue, concentrating on the bacterial reduction effect of the dyes with and without red light irradiation, alone or in combination with frozen storage of the blood, and on long-term testing of the impact of the dyes on the development of the obligate symbiotic bacteria. The objective is to develop an anticoagulant/dye/anti foaming agent mixture to simplify blood collection and processing that will be able to be used under African conditions.

2.5 Pupal Sexing

Recent work at the USDA-ARS, Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, KS 66502, USA using near infrared spectroscopy to characterise wheat grain properties demonstrated that this technique could also identify grains infested by beetles. Further work indicated its potential to identify species, sex and age or some insects, and the system was tested for sexing the pupae of various Diptera, without success.

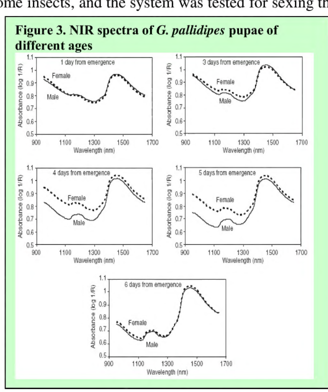


Figure 3. NIR spectra of *G. pallidipes* pupae of different ages

We had investigated the possibility of using NIR spectroscopy for sexing tsetse pupae previously through a contract with the Technical University, Budapest, but although some signals were identified the results were inconclusive and the work was discontinued. As the new system used by the USDA-ARS showed more promise, trials were performed with *G. pallidipes* pupae sent to Manhattan, Kansas, and subsequently Dr Floyd Dowell visited Seibersdorf in February, bringing with him a single

kernel NIR spectrometer with automatic feed, and a separate manual feed spectrometer. The initial trials in Manhattan resulted in a preliminary calibration that achieved better than 85% accuracy, and this preliminary calibration was tested again, and a new calibration was constructed. Individual pupae were measured in the instrument and then held in small pillboxes until emergence so that they could be sexed. Partial regression analyses on the sorted spectra were used to construct the calibrations. A number of pupae were also held and scanned each day until emergence.

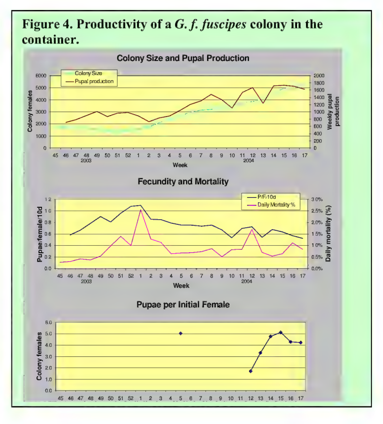
The sequential scanning of pupae until emergence revealed an interesting pattern of change in the spectra (**Figure 3**). Up until 6 days before emergence the male and female spectra are very similar, but about day 5 the absorbance in the range 900–1300nm rose in females and not in males. This rise was subsequently observed in males at about day 3 before emergence so that the spectra once again become very similar. This difference at days 4–5 allows a very high accuracy of sorting, greater than 95% in many samples. It is however possible to construct day specific calibrations able to achieve good levels of separation even for those days when the spectra differ little to the eye. The Unit has ordered one of the single kernel NIR-spectrometers for delivery in 2005, and the work will be continued with Dr Dowell once this arrives.

2.6 Tsetse Rearing Container

Initial testing of the tsetse rearing container (ANNUAL REPORT 2002, 2003) was completed in 2004. One test unit of 1700 female *Glossina fuscipes fuscipes* was introduced into the container in week 44 to test for possible toxicity and problems with the air handling system. No problems were observed, so flies emerging from the initial test unit were retained in the container to create new units from week 51.

The performance of the colony remained good throughout the test period (**Figure 4**). Daily mortality averaged 0.86% (normal range 0.7–1.2%) and fecundity a very good 0.74 p/f/10d (normal range 0.55-0.65). Overall unit performance, as measured by pupae per initial female (PPIF), was also good, averaging just over 4.4 (normal range 3.5–4.5). The low PPIF of unit 51, which is plotted in week 12, the week in which it finished, is not significant as this unit contained only 28 flies and so is subject to large uncertainty. Rearing was discontinued in week 17.

Figure 4. Productivity of a *G. f. fuscipes* colony in the container.



These results show that under the conditions in Seibersdorf, the container is suitable for tsetse rearing. The container was used for fly holding, feeding, pupal incubation, and sex separation, but blood preparation, feeding tray cleaning and sterilization were done in the main building. The container would be suitable for the operation of TPU3, having enough room for 14 single frames plus a feeding trolley, and by combining the container with further container units the necessary facilities for blood storage, preparation and cleaning could be provided. If such a system were developed, it would allow for the rapid development of a rearing facility of any desired size, that would also be easy to relocate, either to keep ahead of an eradication front or to a new project area.

3. Genetic Sexing and DNA Analysis in Medfly

The VIENNA 8 strain, incorporating an inversion and having increased productivity, has continued to be supplied to rearing facilities and laboratories around the world and no further development of improved strains using classical methods has been carried out. The basic components of the strain are maintained and the GSS can be synthesized as required. A small colony is maintained by in the rearing facility in order to provide the necessary training for fellows.



A more detailed analysis of many aspects of the medfly genetic sexing strains (GSS) incorporating the temperature sensitive lethal mutation has been completed in order to provide end-users with more advice in terms of improving efficiency during mass rearing. Detailed information is presented on productivity, temperature sensitive period, viability, speed of development and temperature sensitivity of individuals carrying one copy of the mutation These data are used to improve temperature treatment schedules in mass rearing facilities using GSS for their programmes.

Molecular markers can be very useful in SIT programmes in order to differentiate released flies from wild flies. A class of markers that are in use for medfly are derived from the mitochondrial DNA molecule using restriction site analysis. In order to identify a marker in a release strain it is important to know the genetic variation in this molecule in the target field population. Two studies, one in Madeira, Portugal and one in Mendoza, Argentina are reported.



An alternative approach to marking flies for release is to transform them with a genetic construct that expresses a fluorescent protein. Several strains of medfly have now been produced in the Unit and under investigation as to their suitability in terms of stability and fitness. The genetic construct has been introduced into the VIENNA 8 genetic sexing strain for the evaluation.





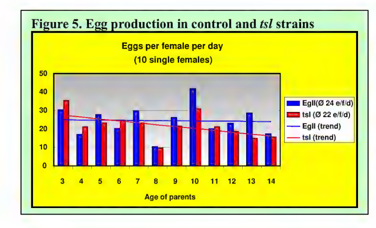
3.1 Genetic Sexing

Current medfly genetic sexing strains (GSS) contain two essential components; the Y-autosome translocation and the temperature sensitive lethal mutation (*tsl*). The *tsl* is used to eliminate the females by raising the temperature during egg incubation. In this report some of biological properties of this mutation are described with special reference to the practical application of GSS. The *tsl* mutation was discovered in a *white pupae* (*wp*) strain originating from Israel. Probably due to the crossing scheme used to detect the *tsl*, both mutations are closely linked on the right arm of chromosome 5. At some stage there was a discussion as to whether the *wp* mutation should be removed. However, today it is clear that this second, easily scoreable phenotypic marker close to the *tsl* has very important practical benefits. For example, without *wp* it would not be possible to implement the Filter Rearing System (FRS). The biochemical function of the *tsl* mutation is unknown. However, from the data presented below it has to be assumed that the gene is involved in central aspects of medfly embryonic, larval and pupal development as well as in adult viability.

3.1.1 Productivity of the tsl strain

To assess the productivity of a strain homozygous for the *tsl* mutation, 10 single pair matings were set up and the number of eggs was determined daily starting three days after the emergence of the flies. As **Figure 5** shows, the number of eggs/female/day (e/f/d) for the *tsl* strain is only slightly reduced (mean=22 e/f/d) as compared to a wild type strain (EgII, mean=24 e/f/d). This result was reproduced on several occasions. It has to be stressed, however, that egg production of homozygous *tsl* females is strongly influenced by the room temperature, i.e. as will be discussed later also the adults are temperature sensitive. Experience has shown that under mass rearing conditions, only 15-16 e/f/d can be obtained while in the more relaxed filter rearing cages values very close to the ones mentioned here are possible. The second component of productivity is the overall viability. As described in ANNUAL REPORT 2002, a homozygous *tsl* strain produces on average 690 adult offspring per 1000 eggs, i.e. even at relatively optimal conditions a *tsl* strain produces 170 adults less than the wild type strain EgII. In a GSS the reduced viability leads to a reduced number of females while the number of males is not affected by the *tsl* mutation.

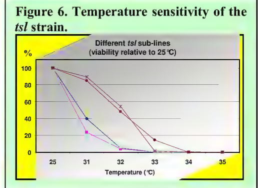
Figure 5. Egg production in control and *tsl* strains



3.1.2 Standard test for temperature sensitivity

To determine the temperature sensitivity of the *tsl* strain parallel samples of 100 eggs, in most cases collected for 24h, are placed on a strip of black filter

Figure 6. Temperature sensitivity of the *tsl* strain.

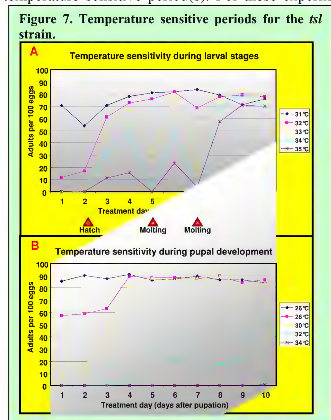


paper. After counting the eggs, the filter paper is put on top of the larval diet in a 9cm Petri dish. The closed Petri dish is incubated at a standard set of temperatures ranging from 31 to 35°C in comparison to a control at 25°C. After an incubation of 24h the Petri dishes are returned to 25°C. **Figure 6** shows the results obtained with different *tsl* sub-lines. It can be seen that an incubation at 34°C leads to complete lethality. For this type of test procedure it is important that the larval diet and the filter paper stay wet during the treatment as drying out leads to reduced lethality.

3.1.3 Temperature sensitive period of the tsl

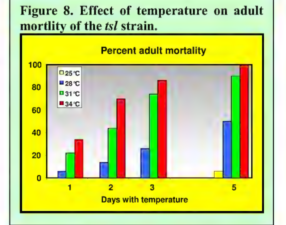
For the use of the *tsl* mutation it is important to accurately determine its temperature sensitive period(s). For these experiments eggs were collected only for 1h. The eggs were temperature treated for 24h during different days of larval and pupal development. **Figure 7A** shows that the temperature sensitivity is highest during the embryonic stages. However, there are two additional temperature sensitive periods that coincide with the molting from first to second and from second to third instar larvae respectively. It is not known whether there is a direct biological connection between the *tsl* mutation and the molting. Also the pupal stages are sensitive to elevated temperatures (**Figure 7B**). However, also wild type pupae are temperature sensitive, especially during the first days of pupal development, although to a lesser extent than a *tsl* strain. These tests clearly demonstrate that the larval and pupal rearing procedures have to take into account that the females of a GSS are more sensitive to temperature than the females in a standard bi-sexual strain. Appropriate measures to reduce the heat load in the larval diet include a special design of the larval trays as well as better air conditioning/handling.

Figure 7. Temperature sensitive periods for the *tsl* strain.



To test the temperature sensitivity of adults, cages were set up with 50 couples and incubated continuously at several different temperatures. **Figure 8** shows that already a treatment with 28°C leads to an increase in adult mortality even after only 1 day of incubation. In the control cages with a wild type strain none of the temperatures used here leads to additional

Figure 8. Effect of temperature on adult mortlity of the *tsl* strain.



mortality. Consequently, the rooms with the adult colony have to be temperature controlled carefully to avoid the loss of females. In addition it was observed that homozygous *tsl* flies respond to inappropriate temperatures by assembling in the bottom of the cage. This obviously leads to reduced egg production. As much as the sensitivity of the adults is a complication for the mass rearing, it is also a benefit for the release as homozygous *tsl* individuals (in a sexing strain the females) will not be viable in the field at elevated temperatures. This applies to flies escaping from the factory as well as to the few remaining females in the release material.

3.1.4 Viability and speed of development of the tsl strain at different temperatures

The *tsl* mutation was also analyzed with respect to the speed of development at 25°C in comparison with different incubation times with a sub-lethal temperature (29°C). Eggs were collected for 1h followed by an incubation at the elevated temperature for 2, 3, 4, 6 and 8d. In the 2d treatment only eggs are incubated while the 8d treatment covers nearly the entire development from egg to third instar. Egg hatch and the number of pupae and adults were determined daily. **Figure 9A** shows that an elevated temperature during the embryonic development leads to an earlier hatching of the eggs by ca 1d. Regardless of the length of the temperature treatment, pupation and adult emergence occur more or less on the same day as in the 25°C control except that emergence is more spread out and a considerable proportion of the flies emerge later than in the control. The consequences for the viability caused by the different treatments are shown in **Figure 9B**. None of the treatments affects egg hatch or the number of pupae. However, the temperature treatments do affect the number of emerged flies. In all treatments emergence is reduced to roughly 200 adults/1000 eggs. Potentially the 8d treatment has a slightly more severe effect. However, in general already the treatment of the embryonic stages (2d treatment) is sufficient to cause a late lethality, i.e. reduced emergence. Fortunately, high temperatures during the egg stage do not present a problem in mass rearing as the eggs are maintained under conditions where the temperature is controlled easily.

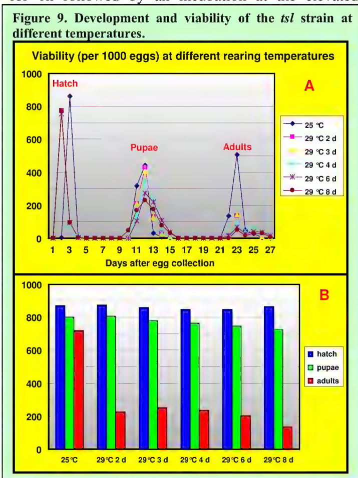
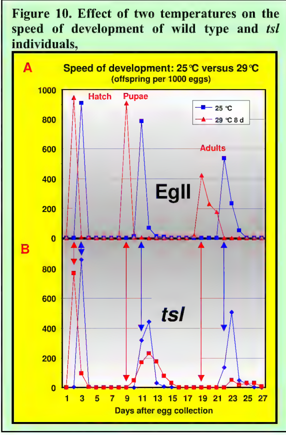


Figure 9. Development and viability of the *tsl* strain at different temperatures.

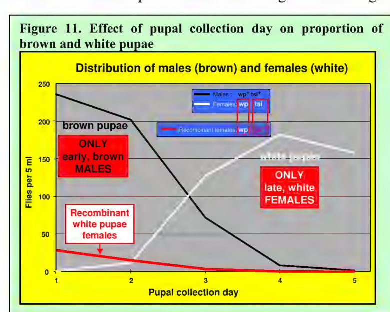
In addition, the influence of extended exposure to sub-lethal temperatures was analyzed in comparison to a wild type strain. A treatment during the first 8d of development at 29°C leads to a significant increase in the speed of development (**Figure 10A**). In comparison to the control (25°C), eggs hatch 1d earlier, pupation occurs 2d and emergence 3d earlier, i.e. the embryonic, the larval and the pupal period are reduced by 1d each. In case of the homozygous *tsl* strain (**Figure 10B**) the development from egg to adult at 25°C takes roughly 1 day longer than the wild type strain. The treatment with 29°C reduces only the embryonic period by one day while the remaining development is unaffected. Emergence might even be slightly delayed.

Figure 10. Effect of two temperatures on the speed of development of wild type and *tsl* individuals,



This has important implications for the maintenance of a *tsl*-based GSS. The length of the larval period of males and females is different and will be strongly influenced by the rearing temperature. The difference in the time of pupation appears to be a good indicator for the temperatures during larval rearing and poor rearing conditions, such as the excess of heat in the larval diet, become visible as increased difference in the pupation time of tsl^+ males and *tsl* females. Temperature control during larval rearing can be particularly difficult if extra biological heat is generated by contaminants (bacteria, fungi, Drosophila) or by heat building up due to the consistency of the diet or the aggregation of larvae. **Figure 11** shows as an example the distribution of male (brown) and female (white) pupae during the five days of pupal collection. The first collection consists virtually of only males while the last collection contains only females. In principle this separation could be used to produce only males for release while the females could be used to rear parasitoids. An additional application is the loading of production cages with a

Figure 11. Effect of pupal collection day on proportion of brown and white pupae



distorted sex ratio without the need for a pupal sorter, i.e. more females than males are used to increase the egg production. Combining the appropriate pupal collections allows the sex ratio to be adjusted in the production cages. The surplus of males from the first pupal collection can be added to the release material.

The distribution of male and female pupae during the pupal collections is also important for maintaining the integrity of the sexing system in a FRS because there is one class of recombinants that cannot be detected in the FRS where the removal of recombinants is based on the link between sex and pupal color. Only males from brown pupae and females from white pupae are used to set up the next generation while males from white pupae (type-2 recombinants) and females from brown pupae (type-1A recombinants) are discarded. The third type is the result of recombination between *wp* and *tsl* leading to *wp tsl*$^{+}$ females (type-1B recombinants). These female recombinants emerge from white pupae and can only be separated from the non-recombinant females via their speed of development. As they are heterozygous for the *tsl* mutation their development from egg to pupae is identical to that of the brown pupae males. Consequently, they are primarily found together with the males in the earlier pupal collections. If the next generation in the FRS is set up only with brown males from an early collection and white females from a late collection the type1-B recombinant females are excluded. The effectiveness of such a selection was demonstrated very dramatically in an SIT programme in Australia. During the mass rearing type-1B recombinants accumulated rapidly. This became apparent by the continuous increase in the number of white pupae that survived the temperature treatment (**Figure 12**). However, when the selection described above was introduced into the filter rearing the number of type-1B recombinants was reduced significantly.

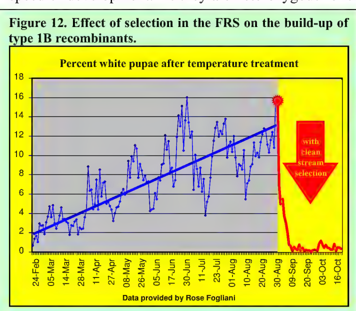


Figure 12. Effect of selection in the FRS on the build-up of type 1B recombinants.

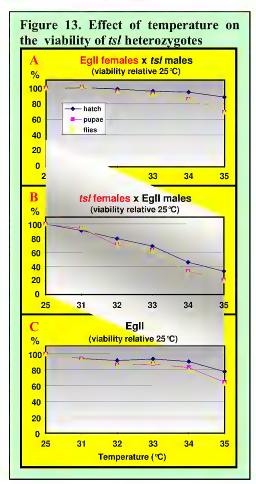
3.1.5 Temperature sensitivity of heterozygous tsl individuals during the egg stage

The experiments described so far were done with the homozygous *tsl* strain and in most cases the eggs were treated immediately after collection. However, during the analyses of *tsl*-based GSS it was noted that such an early treatment also affects the viability of the GSS males despite the fact that these are heterozygous for the *tsl* mutation, i.e. the question is raised whether the wild type allele of the *tsl* is completely dominant. Treatments during later stages, i.e. larvae, pupae and adults, did not cause more male lethality than expected from a wild type strain. To investigate the cause for the male temperature sensitivity during the egg stage, the two reciprocal crosses that lead to the *tsl/tsl*$^{+}$ genotype were set up. After collection for 24h the eggs produced by these crosses were treated directly for 24h with the standard set of

temperatures. If the cross was set up with wild type females and homozygous *tsl* males no significant lethality was observed (**Figure 13A**) comparing the results with a wild type strain (**Figure 13C**). However, if the eggs produced by the reciprocal cross were tested, the egg hatch is reduced significantly (**Figure 13B**). Pupation is affected only moderately and only at the two highest temperatures. At 34°C percent pupation is reduced to 72% relative to the value at 25°C and at 35°C to 60%. Emergence is unaffected.

It has to be concluded that this *tsl* mutation exhibits a maternal effect. During the early stages of development the embryo's own genome is inactive and it apparently utilizes a *tsl* gene product that is pre-produced by the mother. Consequently, during these early stages of development the embryo is dependent on the genotype of the mother and in a GSS the mother is homozygous for the *tsl*. Only later, after activating its own genetic material, are the heterozygous *tsl/tsl*$^{+}$ GSS males protected against elevated temperatures. Based on these findings the temperature treatment regime was modified, i.e. after collection the eggs are maintained at least 24h at 25°C before the treatment is applied.

Figure 13. Effect of temperature on the viability of *tsl* heterozygotes



3.2 Molecular Markers

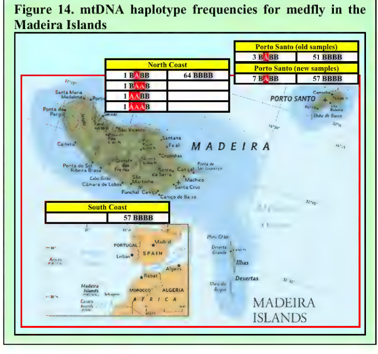
For SIT programmes it is important to be able to distinguish the endogenous population from either invasions or from released flies. The mitochondrial DNA (mtDNA) can be used for this purpose. It is a circular molecule of ca 15kb length and 4 polymorphic restriction sites have been found so far: EcoRV, XbaI, MnI and HaeIII (see ANNUAL REPORT 2001). The haplotype with respect to these enzymes is always given in this order whereby the letter A stands for presence and the letter B for absence of the site. In case of MnlI the different letters refer to different restriction patterns generated rather than to presence/absence of a site. Fragments containing the variable site are PCR amplified using appropriate primers, digested with the respective enzyme and followed by separation on agarose gels.

3.2.1 MtDNA haplotypes in Madeira

The haplotype of the Madeira population was determined on several occasions. Originally the population had the haplotypes BBBB. However, in 2001 B. McPheron (Pennsylvania State University) found that 30% of the flies from Madeira and the neighbouring island Porto Santo showed an AABB haplotype (see ANNUAL REPORT 2001). As a follow-up to this finding a more detailed analysis was undertaken. Flies were collected from Porto Santo and the north and the south coast of Madeira, respectively. All flies collected from the south coast (57 flies) were BBBB.

In contrast, of the 68 flies from the north coast 4 flies (= 6%) showed different haplotypes (**Figure 14**). However, of these only 1 had the haplotype AABB as described by McPheron to occur in 30% of the population. A similar result was obtained with the flies from Porto Santo, i.e. in two separate samples of 54 and 64 flies respectively, 3 and 7 flies with a BABB haplotype were found. Our findings differ from those by McPheron: a) in addition to the standard BBBB we detect in the Madeira (north coast) and the Porto Santo populations four different haplotypes and only one of these is AABB, b) the overall frequency of non-BBBB types is much lower than described by McPheron. Currently, there is no explanation for these discrepancies. One possibility could be that the sampling sites were very different. However, that would mean that in different parts of Madeira and Porto Santo discrete populations with a very different haplotype mix must exist.

Figure 14. mtDNA haplotype frequencies for medfly in the Madeira Islands



The fact that Madeira had a uniform BBBB haplotype was considered to be remarkable because this haplotype was not found anywhere in the Mediterranean region. This includes mainland Portugal and Spain where a mixture of AAAB and AABB haplotypes were found. Also the available data from African samples show that the BBBB haplotype is relatively rare. We have received samples from the African country closest to Madeira, i.e. Morocco, and these flies will be analysed soon. High frequencies of the BBBB haplotype are only found in Venezuela, Brazil and Argentina (see below).

It is difficult to determine where the new haplotypes on Madeira/Porto Santo come from. On the one hand it could be possible that the original data are misleading, for example due to small sample size, i.e. the rare non-BBBB types, present in 6 to 8.5% of the flies, were simply overlooked. Alternatively, the new haplotypes could represent new introductions. Several African countries have haplotype distributions similar to the new ones found on the islands. Mediterranean countries are less likely as a source because all flies examined by McPheron have an AAxx haplotype. However, a recent study with flies from Spain showed that all 9 flies analysed are of the BAxx haplotype (McPheron: mixture of AAAB and AABB). The sexing strain used in Madeira is based on a wild type strain from Egypt. This strain carries the mtDNA haplotype AAAA which was detected so far only in Greece and in Central America. None of the new haplotypes observed in Madeira and Porto Santo is AAAA

and consequently it can be concluded that their occurrence is not caused by an accidental release of fertile GSS females.

3.2.2 MtDNA haplotypes Argentina

The distribution of haplotypes in Central and South America according to McPheron is shown in **Figure 15** (the numbers in front of the haplotypes refer to the number of flies tested). Based on this map the following regions can be distinguished: a) Central America (Guatemala, Costa Rica and Panama): all three countries analysed show an AAAN (N=not determined) haplotype which sets them clearly apart from the South American countries. Two strains (Petapa and Toliman) established with wild material from Guatemala are AAAB. It was reported that in Costa Rica and Panama also the AAAA haplotype can be detected, b) Western part of South America (Colombia, Ecuador, Peru): predominantly AABN with a low frequency of AACN. A strain from Chile (bi-sex mass rearing) will be analysed soon, c) Northern and eastern part of South America (Venezuela and Brazil): Predominantly BBBN with rare AABN cases, and d) South-eastern part of South America (Argentina): a mixture of BBBN and AACN.

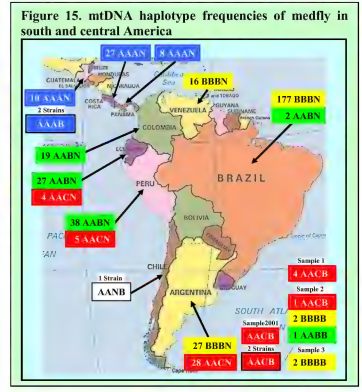


Figure 15. mtDNA haplotype frequencies of medfly in south and central America

The published analyses were done only with the first three enzymes. However, later tests with all four enzymes suggest that with the exceptions of the two countries mentioned above, all populations are B for the fourth enzyme. Several samples were received from Argentina to determine the mtDNA haplotype. Three older samples were uniform AACB. Three samples analysed recently are more diverse, i.e. one sample was uniform AACB, one was uniform BBBB and one was a mixture of AACB, BBBB and AABB. Except for the latter haplotype, which was not detected in this region before, the overall haplotype distribution is the like the one typical for Argentina. The GSS used in Argentina are either AAAA or AAAB. None of the flies analysed showed this haplotype and consequently an accidental release of fertile females can be excluded.

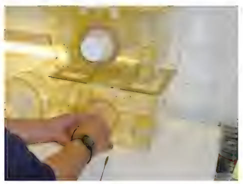
4. Fruit Fly Rearing and Quality Control

Current irradiation practice for in medfly, Ceratitis capitata, *SIT operational programmes is to place pupae, two days before emergence, into polythene bags, allowing pupal respiration to generate anoxic conditions and then irradiate the pupae inside the bag. This procedure is not well standardized and consequently the degree of anoxia during radiation can vary resulting in different responses to irradiation. In an attempt to improve quality control procedures for this component of sterile male production work was carried out to monitor oxygen concentration inside the bag following different times and temperatures of pupal storage. Dr. David Nestel spent two weeks in the Unit to carry out this work. Storage times and temperatures were identified that provide maximum levels of anoxia and which will be incorporated into quality control protocols.*



The Bactrocera oleae *colony is now at a size of about 50,000 individuals and is providing material not just for activities in the Unit but also for colleagues in many institutions. An alternative to the very expensive cellulose used in the larval diet has already been identified and evaluated successfully. In addition, wild genetic material from a population in Spain has been backcrossed into the long established laboratory colony. It is planned that in 2005 field cage mating studies will be carried out with this hybridized strain. In addition alternatives for another expensive larval dietary component will be evaluated.*





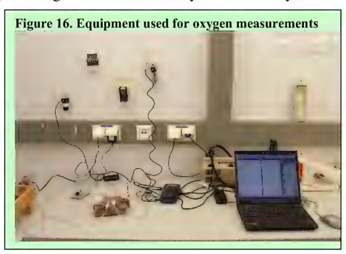
Genetic sexing strains for two other fruit fly species have now been received in the Unit, both are based on white pupal colour mutations. The two species are Bactrocera dorsalis *and* B. cucurbitae. *For both these species SIT programmes are in place and in collaboration with colleagues from Hawaii, quality control and stability evaluations will be carried out. This will also involve detailed analysis by the genetics group of the behaviour of the two translocations used to construct the GSS. In addition mating compatibility will be assessed between the* B. dorsalis *GSS and a field population from Thailand.*

4.1 Irradiation Atmosphere for Medfly Pupae

Current practice in medfly operational SIT programmes is to irradiate pupae, 2 days before emergence, in plastic bags under anoxia, however the degree of anoxia and its relationship to temperature and storage time is not known. In order to better standardise the irradiation conditions, David Nestel, a consultant from the Volcani Centre, Israel spent some time in the Unit to conduct experiments to measure the quantity of oxygen inside of the plastic bags normally used for pupal irradiation.

Between 400 to 500ml of pupae of the appropriate age from GSS VIENNA 8 were placed inside 4.5L polyethylene bags that are commonly used in many mass-rearing facilities. Bags were hermetically sealed, leaving an empty space of 3-5cm above the pupae. Before sealing, bags were perforated in two places using luer-lock connectors which permitted air to be circulated by a pump and the oxygen content of the closed system to be monitored (**Figure 16**). The depletion of oxygen over time due to respiration of the pupae was registered by a data-logger that generated one measurement per minute. Monitoring was discontinued when the oxygen content was close to zero over a period of 10min. This time was considered as "maximal hypoxia". Experiments were conducted at 24°C, except when specified otherwise. Radiation was carried out in a Nordion Gamacell-220 and the dose verified using Gafchromic dosimetry. After irradiation, routine quality control tests were carried out on the pupae and the emerging flies that included field cage competitiveness tests.

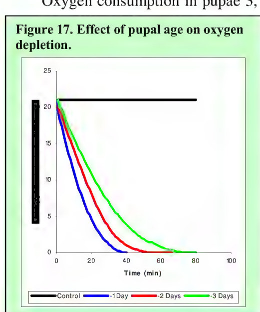
Figure 16. Equipment used for oxygen measurements



4.1.1 Rate of oxygen consumption in sealed bags as affected by pupal age

Oxygen consumption in pupae 3, 2 and 1d before adult eclosion was measured at 24°C as described above. There were 2-3 replicates/treatment and a control bag with no pupae was also included. Rate of oxygen consumption was determined from the oxygen decay curves 10 min after hermetically sealing the bags. **Figure 17** shows the effect of pupal age on the consumption of oxygen by pupae in a close air system. It can be seen that the older the pupae, the faster the consumption, and decline, of oxygen within the bag. The rate of O_2 consumption 10 minutes after the bag was sealed was significantly faster in pupae 1d before adult eclosion than in pupae 2 and 3d before eclosion (F=20.9, df=2,6, P<0.01). Similarly, the time needed by the pupae to reach maximal hypoxia in the bag

Figure 17. Effect of pupal age on oxygen depletion.



was significantly longer in pupae 3d before adult eclosion than in pupae 2 and 1d before eclosion (F=9.3, df=2,6, P<0.05).

4.1.2 Attainment of hypoxia as a function of pre-irradiation incubation temperature

Polyethylene bags were filled with 4.5L of pupae, 1 day before emergence. Pupae were then incubated for 1h with the bags open at two temperatures, 16°C and 24°C. After one hour, bags were sealed and immediately connected to the closed airflow system to measure oxygen consumption at 16°C or 24°C. Pupae incubated at 24°C pre and post sealing consumed O_2 faster than pupae incubated at 24°C pre-sealing and then transferred after to 16°C following sealing for PSB incubation (**Figure 18**). Similarly, pupae incubated before-sealing at 16°C and then transferred to 24°C consumed O_2 faster than equivalent pupae left permanently at 16°C. The average times needed for pupae to reach maximal hypoxia conditions when incubated at the 4 temperature combinations were: 57.5min for 24°C/24°C; 87min for 24°C/16°C; 98min for 16°C/24°C and 148min for 16°C/16°C.

Figure 18. Effect of temperature on oxygen depletion

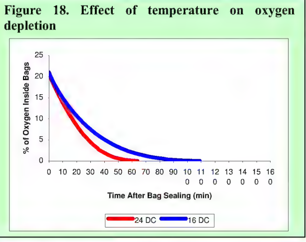
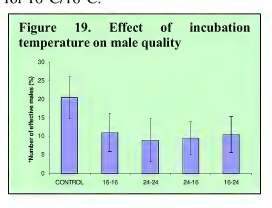


Table 2. Effect of different temperatures on quality control parameters.				
Treatment	**Mean pupal wt ± sd (mg/pupae)**	**Mean # no. pupae/5ml ± sd**	**Mean. % eclosion ± sd**	**FAI (%) ± sd**
24 °C→24°C	8.65±0.07	285±1	87.7±9.1	84.8±8.6
24 °C→16°C	8.50±0.28	270±25	83.1±16.5	85.7±10.4
16 °C→24°C	8.70±0.14	285±3	86.7±12.3	86.2±9.7
16 °C→16°C	8.55±0.07	291±13	84.2±13.3	86.0±7.5
Control *	8.75±0.07	298±12	92.7±2.6	91.1±7.5
H	4.1	3.7	1.0	1.5
P	> 0.05	> 0.05	>> 0.05	>> 0.05

Table 2 shows the resultant quality control tests for the four temperature treatments in addition to the control pupae. None of the investigated quality parameters significantly differed between treatments and control. Similarly, incubation temperature protocols did not have any significant effect upon number of effective males on the field but there was a significant effect due to radiation (**Figure 19**).

Figure 19. Effect of incubation temperature on male quality



4.1.3 Effect of relative oxygen concentration on quality

Pupae were irradiated in sealed bags at 15min after sealing (approximately 10% relative oxygen contents in bag), at 30min (approximately 2% relative oxygen content in bag), and at 60min (maximal hypoxia). In addition, pupae in a non-sealed bag were

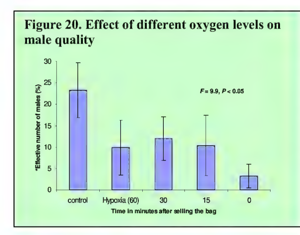
also irradiated. This experiment was replicated two times with different batches of pupae. After irradiation, polyethylene bags were opened and a sample of pupae taken for quality control tests. Non-irradiated pupae were used as a control. **Table 3** shows the effects of irradiation under several O_2 environments in the packing bags on pupal weight, eclosion and percentage of flying males.

None of the treatments have a statistical significant effect upon the quality control parameters (**Table 3**). However, irradiation under full oxygen contents slightly reduced the level of adult eclosion and flight ability (FAI) (**Table 3**). **Figure 20** shows the effect of O_2 environments during irradiation upon number of effective males. Irradiation under full oxygen environment in the packing bag (open bags during irradiation) significantly affected the number of effective males. Irradiation under maximal hypoxia, 2% O_2 and 10% O_2, had comparable number of effective males. Control (non-irradiated pupae), as expected, gave a significantly higher number of effective males than the irradiated pupae.

Table 3. Effect of oxygen concentration on quality control parameters

Treatment	Mean pupal wt ± sd (mg/pupae)	Mean # no. pupae/5ml ± sd	Mean. % eclosion ± sd	FAI (%) ± sd
Control **	8.45±0.40	301±8	89.4±2.3	86.3±0.7
Max. hypoxia	8.40±0.30	298±8	89.2±0.9	82.6±1.8
10% O_2	8.45±0.20	302± 4	89.6±1.8	81.6±4.7
2% O_2	8.50±0.30	303±1	90.9±2.9	84.2±1.9
Full O_2	8.55±0.20	303±11	85.3±6.8	67.4±13.4
H	1.2	0.9	1.5	7.3
P	>> 0.05	>> 0.05	>> 0.05	> 0.05

Figure 20. Effect of different oxygen levels on male quality



4.1.4 Effect of fluorescent dye on oxygen consumption patterns and irradiation effects

Two polyethylene bags were loaded with 500ml of pupae 1d before adult eclosion. One of the bags was treated with 0.75g of Day-Glo® powder. The second bag was used as a control. These bags were sealed and the consumption of oxygen through time measured. After reaching maximal hypoxia, bags were irradiated, opened and a sample of pupae taken for quality control tests. Oxygen decline curves in packing bags of pupae covered with fluorescent dye dust and non-dyed pupae were very similar. Rate of O_2 consumption by non-dyed pupae at 10min after bag sealing was -0.857% O_2/min, while that for dyed pupae was -0.870% O_2/min. Time to reach maximal hypoxia in dyed pupae was 58min, while that for non-dyed pupae was 60min. Pupal weight, no. of pupae in 5ml, % eclosion and flying ability was very similar between the two treatments, and comparable to the non-irradiated control.

4.1.5 Conclusions

These studies clearly showed that O_2 contents inside sealed bags loaded with pupae steadily declines through time. Time needed for the attainment of maximal hypoxia was dependent not only on the age of the pupae, but also on the temperature at which pupae are maintained. These results, thus, strengthen the fact that the rate of

O_2 consumption is strongly dependent upon the rate of metabolic activity; metabolic activity increasing significantly in pupae approaching adult eclosion. Incubation temperature both pre and post sealing had a strong effect upon the consumption of O_2 by pupae. As expected, incubation at 24°C accelerated the consumption of O_2 and the metabolism of pupae; while 16°C had a depressing effect both upon metabolism and O_2 consumption. While temperature manipulation prior to irradiation and attainment of hypoxia affected the rate of O_2 consumption, these incubation protocols did not have significant effect on total male quality expressed as number of effective males. In contrast, irradiation in air had an important effect upon mating activity and pupal quality. These results support the hypotheses that low O_2 tension in pupal tissue reduces the formation of toxic free radicals and peroxides during irradiation. Regardless of the mechanism by which O_2 atmosphere damages the quality of irradiated fly, the present study confirmed the fact that hypoxic environments during irradiation enhance the mating performance and quality of sterile pupae.

In practical terms, this study demonstrated that sealing bags, and keeping pupae for approximately 1.5 or 1h at temperatures of 16 or 24°C respectively, is enough to create a hypoxic environment inside irradiating and packing bags. It also demonstrated that regular dying procedures do not seem to affect the ability of pupae to exchange gases and consume O_2 inside bags.

4.2 Olive Fly Rearing.

The primary requirement for mass rearing of the olive fly (*Bactrocera oleae*) is the identification of larval diet of high efficiency and low cost. The first report of artificial diet for olive fly was published in 1963; from this data several attempts were reported in order to identify economic diet for development mass-rearing protocols. However these studies were conducted 10 to 20 year ago and some of the ingredients that were evaluated have been discontinued or produced by different companies with different specifications. It is therefore necessary to identify possible ingredients that are available on the market to reassess their use for mass rearing of olive fly. On top of that the current reference larval diet for olive fly developed at Democritos Research Centre in Athens includes in its formulation as bulking agent an expensive cellulose powder from Schleicher-Schüll®, Germany which has now been discontinued.

Several cellulose powder replacements have now been tested but only one was identified that has the characteristics to provide the physical and chemicals diet characteristics demanded by olive fly larvae, low cost, availability at industrial level, acceptable production levels and produce olive fly pupae of high quality. In the present report the result are presented that were obtained with a samples of cellulose powder obtained fro Mikro-Technik GmbH & CO. KG, Germany cellulose type 402-2b (MT402-2b) compared with the diet prepare with Schleicher-Schull cellulose used as control in terms of production, quality and cost of diet ingredient to produce 1 million of pupae. The formulation of the liquid diet that was used for the preparation of both diets is shown in **Table 4**. **Table 5** shows the production and quality parameters for both types of cellulose. On average Schleicher-

Table 4. Liquid basal diet to prepare olive fly larval diet

Ingredient	%
Water	54.46
Yeast	7.43
Soy Hydrolizate	2.97
Potassium sorbate	0.05
Nipagin	0.20
Sugar	1.98
Olive Oil	1.98
Tween 80	0.74
HCl (2N)	2.97

Schull cellulose showed better production and QC profile during the initial generations. However results from generation F11 under mass-rearing conditions at a colony size of more that 40,000 adults, have shown that the colony has been adapted to the diet formulated with the new cellulose MT402-b and quality control parameters are now not different for both types of cellulose although further work needs to be done to confirm these initial observations. **Figure 21** shows the data for production efficiency at generation 10. The cost per million of pupae only considering the cost of diet ingredients was estimated assuming that each type of diet is able to produce 6 pupae per gram of diet. On the basis of this calculation the cost of one million pupae using SS cellulose is 1836 US$ while the cost for one million of pupae using MT402-b cellulose is 532 US$.

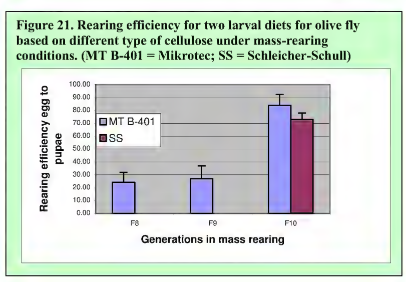
Table 5. Effect of two types of cellulose on quality parameters for olive fly.

	Cellulose type	
Parameter	**Schleicher-Schull**	**Mikro Tech**
Egg hatch	78.2±13.6	78.1±13.6
Efficiency: egg to pupae (%)	50.1±11.5	33.8±33.8
Efficiency: good egg to pupae (%)	60.0±12.6	43.2±26.0
Pupae weight (mg)	5.5±1.1	5.5±0.8
Adult emergence (%)	83.6±3.5	57.4±13.1
Flyers (%)	63.9±3.4	48.3±13.1
Sex Ratio (male %)	56.4±4.8	59.2±6.8

The colony of olive fly that has been successfully established at in the Unit is currently at 50,000 adults and is continually maintained at this level to continue research on diet development and to improve other aspects of mass rearing for this species. We are also now providing this material to other laboratories abroad to facilitate their research in different fields such genetics, biology and control.

In order to further reduce the cost of the larval diet one of the future developments will be to find out a appropriate diet ingredient to replace the soya hydrolysate which is one of the most expensive materials in the diet formulation. Soya hydrolysate is important because it is the source of protein and specific amino acids that are essential for larval development. The aim of these studies will be to identify other available agricultural products that can be used as substitute.

Figure 21. Rearing efficiency for two larval diets for olive fly based on different type of cellulose under mass-rearing conditions. (MT B-401 = Mikrotec; SS = Schleicher-Schull)



4.2.1 Strain refreshment

The current Democritos strain of olive fly has been reared on artificial diet for more than 20 years. Behavioural studies that were conducted in the past between sterile laboratory flies from this strain and wild flies clearly showed that there is a

incompatibility between the two types of fly mainly due to a lack of synchronization on the time of sexual activity. To reassess these behavioural differences studies in collaboration with Mr. Vicente Navarro Lopiz from the Centro de Ecologia Quimica Agricola, Universidad Politecnica de Valencia, Spain, are being carried out. Several shipments of wild pupae have been obtained from Valencia and crosses have been made between females of the Democritos colony and males from Valencia. F1 females were crossed again with wild males from Valencia and this scheme has been followed for the subsequent four generations. Genetic recombination in the female flies should allow effective replacement of the Democritos genome with that of the wild genome. This strain will be used to once again assess the mating behaviour in field cage in comparison with wild flies.

4.3 Larval Density for VIENNA 8

VIENNA 8 is the latest medfly GSS strain and it has been transferred to several operational SIT programmes during 2004. VIENNA 8 has the advantages that all the adjacent-1 individuals are females and are thus killed as embryos by the heat treatment. This means that only genetically balanced male larvae are transferred to the diet thus maximizing its use. In addition, as reported in ANNUAL REPORT 2003, segregation in VIENNA 8 is in favour of alternate balanced individuals and thus this strain is far more productive than all previous GSS. For SIT programmes the balance between quality and quantity is crucial to produce insects of high quality at a reasonable cost. The aim of this investigation was to define and identify the appropriate larval density for male-only production for this strain. The work was carried out by Mr. Jaime Garcia, from the Medfly Eradication Campaign of Chile, when he was a fellow in the Unit.

Eggs from the VIENNA 8 colony were collected and transferred, after thermal treatment, onto larval trays with 5kg of standard wheat brand larval medium. Densities of 40, 50, 60 and 70 eggs/g of diet were tested and 2 trays per replicate for each density were used. The percentage of larvae and pupae recovered and pupal weight suggested an effect of the densities tested (**Table 6**), as at higher densities lower pupal weight were recorded (F=7.30, P>0.01). There was no significant difference found for the percentage of pupae recovery from a given number of eggs seeded. However when the number of flying males/g of diet was analysed, significant differences between densities were found, the higher the density the higher the number of flying insects/g of diet (F=5.21; P>0.01). The analyses of all the parameters suggested that 60 eggs/g of diet can be used as the ideal density as this density produces pupae of acceptable weight and a high number of flying males.

Table 6. Effect of egg density on production of GSS Vienna 8.

	Number of eggs/g diet			
Performance	**70**	**60**	**50**	**40**
Pupae/g diet	14.5±3.3	13.5±1.8	10.3±3.3	9.5±1.4
Flyers/g	12.2±3.1a	11.6±1.9a	9.0±3.2b	8.3±1.7b
Pupal weight	8.0±0.6b	8.2±0.4ab	8.8±0.3a	9.0±0.2a
Efficiency (egg to pupae)	22.0±4.7b	22.4±3.0ab	23.4±6.6a	24.9±3.5a

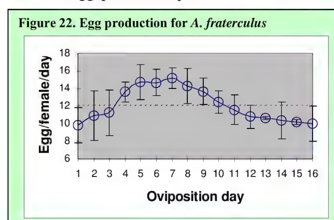
4.4 *Anastrepha fraterculus* Rearing.

For the first time, a standard rearing and QC protocol for *A. fraterculus* has been established and the results obtained during 2004 are described below. Cages are set up with 30,000 pupae and provided with water and adult food, which consists of a

mixture of yeast hydrolyzed enzymatic and sugar (1:3). Cages are held in a rearing room with 25±1ºC, 65±10Rh and a photoperiod of 14:10 L:D. Females lay eggs through the egg collection panel that consists of a plastic frame with a synthetic net (80 mesh nylon monofilament) coated with a thin layer of silicon rubber. The oviposition panel is put on top of the surface of the cage that is sealed with a synthetic net (40 mesh nylon monofilament) to allow the ovipositors to go through and reach the oviposition panel. To avoid dehydration, a layer of water (1cm) was provided inside the oviposition panel. The water is removed every 24h and the eggs collected. The volume of eggs is measured for each cage and three samples of 100 eggs are placed in a Petri dish with moistened sponge to assess hatch. After egg collection, eggs are bubbled in a plastic bottle with water for 48h and then larval trays are seeded to a density of 20 eggs/g of diet. Larval diet for *A. fraterculus* is identical to that of medfly except that wheat bran is replaced by sugar beet bagasse. Larvae are incubated at 25°C for 10 days after which time they start leaving the media and pupate in sawdust placed in a pan below the trays. For 3 days, larvae and pupae were collected every 24h. After 48h the pupae are separated by sieving, the volume measured followed by incubation in a tray in a dark room at 20°C and 80%Rh. Two days before emergence, three samples of 5ml of pupae are weighed to determine individual weight and density of pupae/ml. These samples are kept to assess adult emergence and sex ratio. To determine production efficiency the % recovery of pupae for a given number of eggs was determined for each replicate. In the colony, the number of eggs laid per female in each egg collection day was estimated.

The egg production profile over the lifetime of a cohort of females in small scale experiments is shown in **Figure 22**. The oviposition period lasted about 16 days and peak oviposition was observed between 5 and 8 days of age. For mass rearing the average number of eggs throughout 2004 averaged 12±2.5 eggs/female/day which seems to be a little lower than the results obtained in small-scale experiments.

Figure 22. Egg production for *A. fraterculus*



The QC data for the rearing is shown in **Table 7**. Egg hatchability was low and showed a high variation (78.4±14.4%). For egg density of 15 eggs/g of diet, the average of recovery was 40.0±14%, but this value only represented the larva that crawled out from the diet as it was estimated that around that 30% of the larvae remain and pupate inside of the diet. These data show that the diet based on sugar beet bagase is suitable for rearing this species but method needs to be developed to recover the larvae that remain in the diet. The quality of the material produced (**Table 7**) in terms of pupal weight, adult

Table 7. Production data for *A. fraterculus*

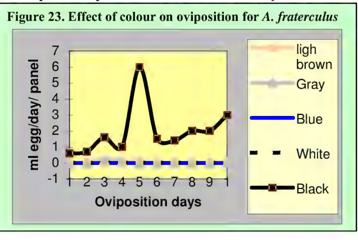
Parameter	Mean±Std
Egg hatch	78.4±14.4
Efficiency egg to pupae (%)	40.0±14.0
Efficiency good egg to pupae (%)	50.0±10.0
Pupae weight (mg)	15.4±1.0
Adult Emergence (%)	85.6±6.9
Flyers (%)	82.4±7.5
Sex Ratio (male %)	46.8±6.2

emergence, sex ratio and adult flight ability was high and can be used as reference to establish QC standards for this species.

4.4.1 Improving egg collection system for A. fraterculus

As show in **Figure 23**, the current average of egg production is 12 eggs/female/day, however data for small-scale experiment using a paraffin cones as egg collection devices has shown that the potential production/female/day is at least 25 eggs. Currently the egg collection system for *A. fraterculus* is based on a silicone panel very similar to the system described for *A. ludens* and *A. obliqua*. However as there are morphological and oviposition behaviour difference between species it is necessary to evaluate the specific requirements of *A. fraterculus* to improve egging cage design and egg productivity. The first step was to evaluate the visual response of the flies to different colours of oviposition panel coated with a fine layer of silicone (0.01mm). Small plastic frames (15.5cm x 26.5cm) with a synthetic net of different colours were tested by exposure to a population of mature and inseminated females (±15000) confined in aluminium frame cage sealed with a synthetic net in the top and sides. Every day from 9.00 to 14.00h, the five silicone oviposition panels were filled with water to prevent egg desiccation and placed on top of the cage to allow oviposition to take place. During the experiment the position of the panels was randomly changed on a daily basis. The results were very clear-cut as the only significant oviposition was found in the black panel. Some eggs were also collected for the grey and brown panels but the volume was almost insignificant (**Figure 23**). Further investigations will be carried out to assess the importance of the thickness of the silicone layer and the minimum oviposition surface necessary for each female in order to optimise the egg production/female/day.

Figure 23. Effect of colour on oviposition for *A. fraterculus*



5. Mosquito SIT



Since its official launch in January 2004, the mosquito SIT programme has seen a rapid expansion of activities, both in the Agency's laboratories in Seibersdorf and in selected field sites. The year was marked by initiation of colonization efforts, studies on radiation biology of the target species Anopheles arabiensis, *initiation of genetic sexing studies and developments of mass-rearing procedures. Impressive progress in field site preparation and vector surveillance studies has been made in Northern State, Sudan, and plans for further mosquito research on La Reunion island were consolidated in a project document. The proposed CRP project on mass-rearing and pre-release biology of* An. arabiensis *was approved and was followed by the drafting of a second CRP proposal on adult mosquito biology. Several new (temporary) staff has become engaged in the above activities, and the project has continued to engage external consultants in many of its activities.*

5.1. Colony Maintenance and Development

In January, an attempt was made to colonize *An. arabiensis* from Northern State, Sudan. Some 1500 larvae were collected from breeding sites close to the Nile River, and these were successfully transferred to the laboratories of the Tropical Medicine Research Institute (TMRI) in Khartoum. Eggs were subsequently shipped to Seibersdorf and a full colony established thereafter (**Figure 24**). The establishment of this colony will now enable research on the biology and mass-rearing of the target species, which, due to its unique location on the fringes of its natural distribution, may display peculiarities not observed elsewhere that may affect development of a SIT approach. Besides this 'Dongola' colony, the KGB strain of *An. arabiensis* from Zimbabwe is maintained to augment these studies and that can also be used for comparative purposes. A second strain from Sudan, the Sennar strain, was obtained and has been shown to display dieldrin resistance levels not seen in either KGB or Dongola. This strain is now utilised to develop a genetic sexing strain based on Y-chromosome translocations of the gene conferring resistance (see also below). Although production of the KGB strain reached 5-7 thousand pupae per day early in the year, space constraints have since led to maintaining smaller-sized colonies.

Figure 24. Pupal collection



5.2 Development of a Classical Female Elimination System.

As only biting female mosquitoes transmit the malaria parasite, their release in the field during a SIT campaign is totally proscribed. Therefore, an efficient sexing mechanism needs to be devised. Amongst the sexing options currently investigated, the development of an *An. arabiensis* genetic sexing strain appears the most promising. This approach will potentially achieve the high sexing efficiency (above 99%) required for safe releases. Toward that end, M. Benedict has characterized markers to be used in the genetic scheme. The dieldrin resistance allele of the Sennar colony was characterized and is the result of a single base mutation in the GABA receptor and will be used as the primary selectable marker for males. In order to create a Y translocation with tight linkage to this gene, an inversion containing the dieldrin resistance locus will be created. Fortunately, a natural polymorphism named *collarless* (**Figure 25**) is present in the laboratory colonies and was demonstrated in crosses performed at the Agency to be loosely linked to dieldrin resistance. This will be used as a second marker to detect such inversions.

Figure 25. The *collarless* mutation



5.3 Germline Transformation as a Supporting Technology for Genetic Sexing

The classical approach to developing a sexing strain for *An. arabiensis* as described above has proven alternatively simple or difficult during previous efforts and its success cannot be guaranteed. Furthermore, the development of a sexing strain can be a long process. For example, the development of the Vienna-8 *Ceratitis capitata* genetic sexing strain necessitated 10-15 years of research. It is thought that recent advances in biotechnology such as mosquito germline transformation may help reduce this traditionally long R&D phase. Therefore, parallel to the efforts to create a conventional genetic sexing strain based on the radiation-induced translocation of a resistance marker to the male-determining chromosome, the mosquito group is undertaking a transgenic approach to genetic sexing. Such work parallels the effort of the WHO-TDR Molecular Entomology Task Force to support molecular research and use newly acquired knowledge towards developing novel strategies for controlling disease transmission.

Figure 26. G. Labbe microinjecting *An. arabiensis* embryos

Our latest efforts have concentrated on improving injection methods appropriate for *An. arabiensis* embryos **(Figure 26)**. Microinjections of *An. arabiensis* embryos are now performed routinely in the laboratory (our new injection protocol can be accessed through the MR4 website. (See:http://www2.ncid.cdc.gov/vector/Tech%20Tips/Microinjection%20Method%20for%20Anopheles%20Embryos%20V2.pdf).

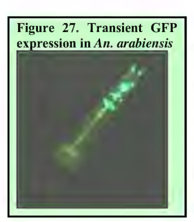
Table 7. Modification of injection protocol resulted in increased embryo survival and improved DNA delivery.

Period of injection	07-09/'04	10-11/'04	01-06/'05
Injection needles[a]	program 5	program 10	program 10
Conc vector/helper plasmids (ng/ul)	500/200	250/100	300/200
Eggs injected	2370	5441	1810
Larvae hatched (%)	2.36%	7.61%	10.44%
Transient GFP expression (%)	ND	23.19%	52.91%

[a]: see weblink regarding parameters used for pulling the quartz needles.

Using this protocol, consistent hatch rates (8-14%) are obtained and transient somatic expression is observed at a high frequency in G0 larvae (**Table 7**) following injection (*piggybac* construct carrying a 3xP3-EGFP expression cassette) (**Figure 27**). Achieving the first successful germline transformation of *An. arabiensis* will require additional efforts to improve the mating efficiency of mosquitoes maintained as small families in order to increase the chances of recovering sufficient numbers of G1 individuals. In parallel, Ms. Labbé is developing the molecular tools necessary to conduct excision and transposition assays to confirm the functionality of *piggybac*-based germline transformation in *An. arabiensis*. Once the first transformation is achieved, our efforts will focus on inserting *piggybac*-

Figure 27. Transient GFP expression in *An. arabiensis*

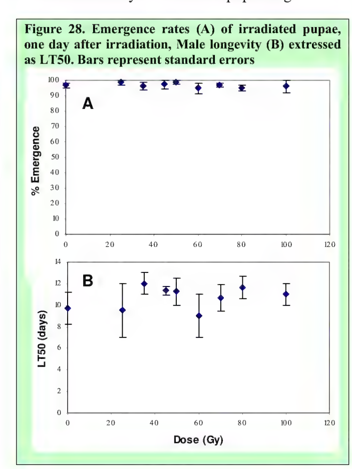


based female-specific constructs into the genome of *An. arabiensis*, to confirm the sex-specificity of transgene (GFP) expression. If this system proves functional in *An. arabiensis,* it will be used to control the conditional expression of lethal (cytotoxic) genes in females allowing their selective elimination and the production of male-only progenies under female-restrictive conditions.

5.4 Radiation Biology of *Anopheles arabiensis*

The development of radiation protocols started in July and continues to focus on the selection of an appropriate life-stage and radiation dose, whilst aiming to safeguard the vigor and competitive ability of sterilised males. Although irradiation of *Anopheles* mosquitoes has been performed in the past on various species, the irradiation of *An. arabiensis* has not been studied in depth. Therefore, base line data had to be gathered on the effects of different irradiation doses on this species. In mosquitoes, both the pupal and adult stage can be irradiated, and experiments to study the effects of irradiation on both stages have to be conducted. It was decided to start with the dose-sterility curve for the pupal stage.

Figure 28. Emergence rates (A) of irradiated pupae, one day after irradiation, Male longevity (B) extressed as LT50. Bars represent standard errors



The mosquito strain used in all experiments thus far is the Zimbabwean KGB strain of *An. arabiensis*, which will later be followed by confirming studies on the Sudanese 'Dongola' strain. Pupae, aged 20-26 hrs, were irradiated in batches using the following doses: 0, 25, 35, 45, 50, 60, 70, 80, and 100 Gy. The effects of irradiation on pupal emergence, induced male and female sterility, and longevity and mating capability of the males was recorded.

5.4.1 Emergence

After irradiation, pupae were placed in individual tubes and the day following irradiation, emergence rates were scored. Overall, emergence rates were high. Irradiation had no negative effect on the emergence rate of the pupae (**Figure 28A**), which was similar to that of non-irradiated controls ($F_{8,28}$=0.63, n.s.).

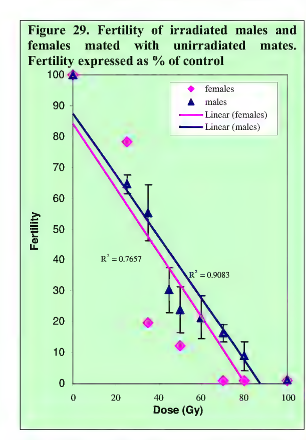
5.4.2 Longevity of males

Each day, mortality in the cage was scored. Irradiation did not influence male longevity, which was similar to control males ($F_{8,28}$=0.94, n.s.) (**Figure 28b**). However, a test that measures longevity under more stressful conditions will need to be developed to assess longevity in a more challenging situation.

5.4.3 Sterility

Sterility was observed by mating the irradiated males and females to non-irradiated mosquitoes of the opposite sex. A human arm was offered on two consecutive days as a blood meal. Mass oviposition in the cages occurred, and hatching rate determined by counting L1 larvae. The level of sterility induced increased with higher doses, and females were more radiosensitive than males (**Figure 29**). For the males, a dose of 100 Gy induced 99% sterility, while 50Gy already induced 80% sterility. Egg production of irradiated females is almost completely inhibited at doses >50 Gy, and of the few eggs that are laid, almost none will hatch.

Figure 29. Fertility of irradiated males and females mated with unirradiated mates. Fertility expressed as % of control



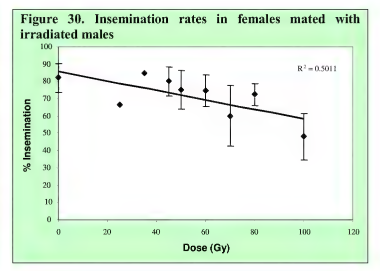
5.4.4 Mating capability

The females that had mated with irradiated males were dissected to score for the presence of sperm in their spermatheca, a measure of mating capability of the males. There is a negative correlation (R^2=-0.50, $p<0.01$; (**Figure 30**) between % of insemination and dose; as irradiation dose increases, males become poorer inseminators. However, as there is quite a large variation between different replicates of the same dose, this will be studied in more depth.

5.4.5 Adults and competition experiments

The irradiation curve for the adult stage is being developed in 2005. In addition, competition experiments will be initiated to test the competitiveness of the males irradiated as pupae or adults. These competition experiments will be conducted in a greenhouse that is currently being constructed in Seibersdorf.

Figure 30. Insemination rates in females mated with irradiated males



5.5 Mass-Rearing Technology

The strategy for mass rearing is to develop all new purpose-designed equipment systems that will be capable of either manual, automatic or semi-automatic operation as appropriate. In order to accomplish the arbitrary goal of producing 1 million sterile males per day, we are developing sub-modules to be constructed in Seibersdorf, each capable of producing 100,000 sterile males per day. Drawing upon previous biological studies and estimates, a modular adult cage was designed that will be tested for both colonization and production purposes (**Figure 31**). Features of this cage include light weight, ease of disassembly and transport, an artificial 'horizon' and resting areas, and tubes into and out of which all mosquitoes and diet are introduced without allowing adult escape. We have calculated that the volume of such a cage would be approximately 1.1m^3. The design will have a footprint of 1x2 meters and 4.2m^2 adult resting area, and a changeable floor permitting continuous operation.



Figure 31. Concept cage design for mosquito colonization and production.

Details of the tube system and capacity testing are expected to be completed during 2005. Materials have been tested for permanent sugar feeding devices and an initial selection of either porous polyethylene or polyester cloth has been made. The latter are also being considered for permanent blood feeding 'membranes.

Studies were conducted in Seibersdorf to develop experimental systems for diet development. These are warranted because previous studies have failed to clearly separate the effects of waste accumulation and larval and diet density. This requires both a new physical setup and diets. We have used protists in a circulating water system in order to achieve uniform water chemistry and diet regardless of larval density. Preliminary studies show that a candidate experimental diet, *Tetrahymena* spp. is easily cultured and provides a suitable experimental diet. However, changes in the circulation system were necessary because of the delicate nature of the organisms.

Adult diets are being assessed for their ability to increase longevity. While longevity may not be the ultimate goal of adult diet supplements, it is a reasonable starting criterion. While 10% sucrose is a standard diet, the group is aware that other research teams have seen improved longevity using vitamins in the meal, particularly antioxidants such as vitamin C. We tested these as well as other sugars and complex sources of carbohydrates (raisins), and the preliminary results suggest that increased longevity can be achieved but the specific diet that consistently produces such results and can be obtained consistently in large quantities has not been determined.

5.6 Field Studies: Sudan

The Government of Sudan has shown a strong interest in the development of mosquito SIT to control if not eliminate malaria from selected areas in Northern State. Early in the year, a national seminar was held in Khartoum, at which all stakeholders (e.g. the National Malaria Control Programme, WHO, etc.) were present. Ministerial support was pledged during the same meeting. Since then, a series of consultant's missions have been conducted to assist the national counterpart (Dr. Badria El-Sayed) with the development of the project's phase I. This phase is aimed at providing base-line information on climatic conditions and mosquito temporal and spatial dynamics.

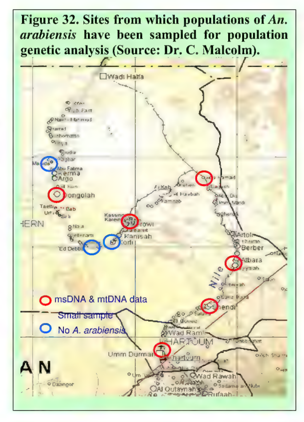


Figure 32. Sites from which populations of *An. arabiensis* have been sampled for population genetic analysis (Source: Dr. C. Malcolm).

A research contract was issued to Dr. Colin Malcolm (Queen Mary University, London) by the Agency's department of Technical Cooperation to study the population genetics of *An. arabiensis* in Northern State. Populations from a variety of areas both in and outside the project area have been sampled and subjected to mitochodrial and microsatellite DNA analysis (**Figure 32**). Such studies elucidate the level at which populations are structured and provide an indication of their homogeneity. Although over the entire length of the River Nile populations do differ substantially, this appears to be limited within the project area (between Dongola and Kareima).

A total of four weather stations were deployed (two in Dongola and two in Kareima) to provide detailed climate temporal humidity, temperature, and wind data. The first full year of automated data collection was recently completed. Data show that extreme weather conditions occur frequently both in terms of low humidity (~ 10% RH) and relatively low temperatures (~ 18 degr. C).

State-of-the-art remote sensing technology and high-resolution images (QuickBird) have been acquired of the areas around Dongola (**Figure 33**) and Merowe/Kareima. Using coupled mobile PDA-GPS systems, mosquito surveillance is currently conducted at monthly intervals in both areas. Local staff has been trained and are now using this technology to create a database that will eventually assist in risk mapping mosquito abundance over the entire bend of the Nile. Such information will ultimately guide pre-release control interventions and assist in planning of the SIT campaign. The collaboration with Dr. Jon Cox of the London School of Hygiene and Tropical Medicine has been instrumental in completing phase I of the project.

The Sudanese government has released substantial financial and equipment resources to the project. Besides all-terrain vehicles it has provided funds for two field stations, which are currently being furbished. These developments are greatly facilitating progress. Two fellowship proposals were awarded through the Agency's Technical Cooperation programme for Sudanese MSc students to undertake part of their studies within the Seibersdorf laboratories. These are currently being undertaken.

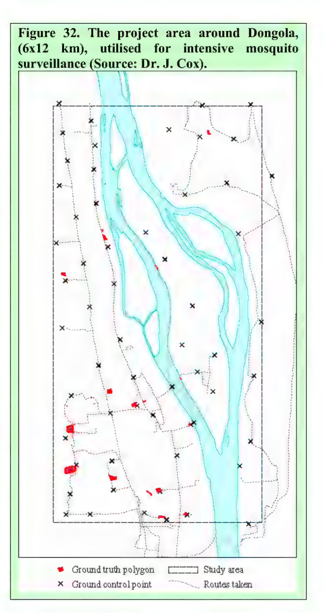


Figure 32. The project area around Dongola, (6x12 km), utilised for intensive mosquito surveillance (Source: Dr. J. Cox).

5.7 La Reunion

The project steering committee for the SIT project on La Reunion island met in Vienna in December. Limited funding from the French government enabled this gathering. The meeting was also attended by officials from La Reunion, and provided the basis for a renewed impetus to further the research necessary for an eventual SIT campaign against *An. arabiensis* on the island. Regretfully, Dr. Romain Girod, who served as the focal point for collaboration on the island, took up a new assignment in Marseille, which temporarily halted activities. Following the meeting, a detailed project document has been drafted and negotiations with the French government are currently underway. It is hoped that research on the island can continue soon, especially considering the huge amount of important information on vector abundance and distribution that is already available that will facilitate progression of the project.

5.8 Additional Collaborations and Developments

A research contract was issued to Prof. Anna-Karin Borg-Karlson of the Royal Technical Institute (KTH) in Stockholm, Sweden. Her research group has extensive experience with the studies on semiochemicals from insects. The group received mosquito eggs from the KGB colony in Seibersdorf and has conducted detailed chemical analyses on the volatiles produced by swarming male *An. arabiensis*. Using coupled GC-MS technology, four volatile organic compounds collected using solid phase micro extraction (MSPE) have been identified that may mediate mating behaviour in this species. Research on this essential component of a mosquito SIT campaign is continuing in 2005.

Following the approval of the first mosquito coordinated research project (CRP) in July, a second consultants meeting was held in Vienna in December to focus on adult mosquito biology. A draft document has been prepared and a full proposal will be submitted in 2005.

Evaluation of critical components of a SIT campaign, such as mating competitiveness or the stability of genetic sexing strains are difficult to study in laboratory cage settings. Following successful completion of the *An. gambiae* life-cycle in contained semi-field environments, it was decided to construct a 250 m^2 facility in Seibersdorf for this purpose. This facility is currently being constructed.

6. Appendices

6.1 Publications

ATKINSON, P.W., D.A. O'BROCHTA and **A.S. ROBINSON** (2004). Insect transformation for use in control. In Insect Pharmacology and Control – a volume in the series "Comprehensive Insect Biochemistry" edited by S.S. Gill, L.I. Gilbert and K. Iatrou. pp 247 263.

BARNES, B.N., D.K. EYLES and **G. FRANZ** (2004) South Africa's fruit fly SIT programme: the Hex River pilot project and beyond. In: Barnes, B.N. (Ed.) Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, Isteg Scientific Publications, Irene, South Africa. pp 367-381.

BILLINGSLEY, P.F., J.D. CHARLWOOD and B.G.J. KNOLS (2005). Rapid assessment of malaria risk using entomological techniques: Taking an Epidemiological Snapshot. In: Environmental change and malaria risk (W. Takken & P. Martens, eds.). Chapter 6, pp 51-67. Kluwer Academic Publishers. Frontis series no. 9.

CACERES, C., J.P. CAYOL, W. ENKERLIN, **G. FRANZ,** J. HENDRICHS and **A.S. ROBINSON** (2004). Comparison of Mediterranean fruit fly (*Ceratitis capitata*: Tephritidae) bisexual and genetic sexing strains: Development, evaluation and economics. In: Barnes, B.N. (Ed.) Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, Isteg Scientific Publications, Irene, South Africa. pp 367-381.

CHANG, C.L., **C. CACERES**, and E. JANG, (2004). Novel liquid larval diet and its rearing system for Melon fly, *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 97: 524-528

DEPINAY, J.M.O., C.M. MBOGO, G.F. KILLEEN, **B.G.J. KNOLS**, J. BEIER, J. CARLSON, J. DUSHOFF, P. BILLINGSLEY, H. MWAMBI, J. GITHURE, A.M. TOURE and F.E. MCKENZIE (2004). A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. Malaria Journal, **3**, 29 (21 pp).

FERGUSON, H.M, B. JOHN, K. NG'HABI, and **B.G.J. KNOLS** (2005). Redressing the sex imbalance in vector biology knowledge. *Trends in Ecology and Evolution*, **14**: 202-209.

FILLINGER, U., G. SONYE, G.F. KILLEEN, B.G.J. **KNOLS** and N. BECKER (2004). The practical importance of permanent and semi-permanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: Operational observations from a rural town in Western Kenya. Trop. Med. Int. Hlth, **9**: 1274-1289.

FRANZ, G. (2004) Genetic sexing. In Encyclopedia of Entomology, ed. J. L. Capinera, Kluwer Academic Press, 957-958.

GOUAGNA L.C., B.A. OKECH, E.W. KABIRU, G.F. KILLEEN, P. OBARE, S. OMBONYA, J.C. BEIER, **B.G.J. KNOLS**, J.I. GITHURE and G. YAN (2004). Seasonality of *Plasmodium falciparum* infection and risk factors for gametocyte carriage in patients attending a rural health centre in Western Kenya. East African Medical Journal, **80**: 627-634.

GBOLADE, A.A., LUKWA, N, KALENDA, D.T., BOËTE, C. and **KNOLS, B.G.J**. (2004). Guidelines for studies on plant-based vector control agents. In: Traditional Medicinal Plants and Malaria (M. Willcox et al., Eds.). CRC Press, FL, USA. pp. 389-408.

IMPOINVIL, D.E., KONGERE, J.O., FOSTER, W.A., NJIRU, B.N., KILLEEN, G.F., GITHURE, J.I., BEIER, J.C., HASSANALI, A. and **KNOLS, B.G.J**. Survival of the malaria mosquito *Anopheles gambiae* on plants from Mbita, western Kenya. Med. Vet. Entomol. **18**: 108-115

KILLEEN, G.F., A. SEYOUM and **B.G.J. KNOLS** (2004) Rationalising historical successes of malaria control in Africa in terms of mosquito resource availability management. Am. J. Trop. Med. Hyg. **71**:87-93.

KNOLS, B.G.J., SUMBA, L.A., GUDA, T.O., DENG, A. L., HASSANALI, A., BEIER, J. C. Selection of oviposition sites by the African malaria mosquito *Anopheles gambiae*. (Diptera: Culicidae) is mediated by semiochemicals of microbial origin. Int. J. Trop. Insect Sci. **24**: 260-265.

MAREC, F., L.G. NEVEN, **A.S. ROBINSON,** M.J.B. VREYSEN, M.R. GOLDSMITH, J. NAGARAJU and **G. FRANZ** (2005). Development of genetic sexing strains in Lepidoptera: From traditional to transgenic approaches. J. Econ. Entomol. 98:119-125.

MARQUEZ, J.G., M.J.B. VREYSEN, **A.S. ROBINSON**, S. BADO and E.S. KRAFSUR (2004). Mitochondrial diversity analysis of *Glossina palpalis gambiensis* from Mali and Senegal. Med. Vet. Entomol. 18: 288-295.

MATHENGE, E.M., OMWERI, G.O., IRUNGU, L.W., NDEGWA, P.N., WALCZAK, E., SMITH, T.A., KILLEEN, G.F. and **KNOLS, B.G.J**. (2004). Comparative field evaluation of the Mbita trap, Centers for Disease Control light trap and the human landing catch for sampling of malaria vectors in western Kenya. Am. J. Trop. Med. Hyg. **70**: 33-37.

MATHENGE, E.M., MISIANI, G.O., OULO, D.O., IRUNGU, L.W., NDEGWA, P.N., SMITH, T.A., KILLEEN, G.F. AND **KNOLS, B.G.J**. Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of *Anopheles arabiensis*, *An. funestus* and culicine species in a rice irrigation scheme in western Kenya. *Malaria Journal 2005*, **4**, 7.

MUKABANA, W.R., TAKKEN, W., KILLEEN, G.F. and **KNOLS, B.G.J.** (2004). Allomonal effect of breath contributes to differential attractiveness of humans to the African malaria vector *Anopheles gambiae.* Malaria Journal **3**, 1. (8 pp.).

MSHINDA, H., KILLEEN, G.F., MUKABANA, W.R., MATHENGE, E.M., MBOERA, L.E.G. and **KNOLS, B.G.J**. (2004). Development of genetically modified mosquitoes in Africa. Lancet Infectious Diseases **4**: 264-265.

NDUNG'U, M., TORTO, B., HASSANALI, A., **KNOLS, B.G.J**., CHHABRA, S. and KERIKO, J. (2004). Larvicidal activity of extracts of five east African Meliaceae plants against *Anopheles gambiae*. Int. J. Trop. Insect Sci., **24**: 311-318.

NIYAZI, N., **C. CACERES**, A. DELPRAT, **V. WORNOAYPORN**, E. RAMIREZ SANTOS, **G. FRANZ** and **A.S. ROBINSON** (2005). Genetics and mating competitiveness of *Ceratitis capitata* (Diptera: Tephritidae) strains carrying the marker *Sergeant*, *Sr2*. *Ann. Entomol.* Soc. Am. 98(1): 119 – 125

OKECH, B.A., GOUAGNA, L.C., **KNOLS, B.G.J.,** KABIRU, E.W., KILLEEN, G.F., BEIER, J.C., YAN, G., and GITHURE, J.I. (2004). Influence of indoor microclimate and diet on survival of *Anopheles gambiae s.s.* (Diptera: Culicidae) in village house conditions in western Kenya. Int. J. Trop. Insect Sci: **24**: 207-212.

ROBINSON, A.S., **G. FRANZ** and P.W. ATKINSON (2004). Insect transgenesis and its potential role in agriculture and human health. Insect Biochem. Mol. Biol. 34:113-120.

SCHOLTE, E-J, **KNOLS, B.G.J**. and TAKKEN, W. (2004). Autodissemination of the entomopathogenic fungus *Metarhizium anisopliae* amongst adults of the malaria vector *Anopheles gambiae* s.s. Malaria Journal 2004, **3**: 45.

SUMBA, L. A., OKOTH, K., DENG, A.L., GITHURE, J., **KNOLS, B.G.J.,** BEIER, J.C. and HASSANALI, A. (2004). Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. J. Circadian Rhythms 2004, **2**: 6.

SUTANTAWONG, M., W. ORANKANOK, W.R. ENKERLIN, **V. WORNOAYPORN** and **C. CACERES**. (2004). The Sterile Insect Technique for control of the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) in mango orchards of Ratchaburi Province, Thailand. In: Barnes, B.N. (Ed.) Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, Isteg Scientific Publications, Irene, South Africa. pp 223-232

In Press

CALKINS, C.O. and **A.G. PARKER**. Sterile insect quality. In "The Sterile Insect Technique: Principles and Practice in Areawide Integrated Pest Management" Eds A. Dyck, J. Hendrichs and A.S. Robinson.

DOWELL, F.E., **A.G. PARKER,** M.Q. BENEDICT, **A.S. ROBINSON**, A.B. BROCE and R.A. WIRTZ. Sex separation of tsetse fly pupae using near-infrared spectroscopy. Bull. Ent. Res.

FRANZ, G. Genetic sexing strains amenable to large scale rearing as required for the sterile insect technique. In "The Sterile Insect Technique: Principles and Practice in Areawide Integrated Pest Management" Eds A. Dyck, J. Hendrichs and A.S. Robinson.

HENDRICHS, J. and **A.S. ROBINSON.** Prospects for the future development of the sterile insect technique. In "The Sterile Insect Technique: Principles and Practice in Areawide Integrated Pest Management" Eds A. Dyck, J. Hendrichs and A.S. Robinson.

IAEA. 2005. Dosimetry System for SIT: Standard Operating Procedure for Gafchromic® film. IAEA, Vienna, Austria.

MEHTA, K. and **A.G. PARKER.** Sterile insect technique: Dose optimization, dosimetry and irradiation. Florida Entomologist.

PARKER. A.G. Mass rearing for sterile insect release. In "The Sterile Insect Technique: Principles and Practice in Areawide Integrated Pest Management" Eds A. Dyck, J. Hendrichs and A.S. Robinson.

ROBINSON, A.S. The genetic basis of the sterile insect technique. In "The Sterile Insect Technique: Principles and Practice in Areawide Integrated Pest Management" Eds A. Dyck, J. Hendrichs and A.S. Robinson.

SCHOLTE, E.-J., **KNOLS, B.G.J**. and TAKKEN, W. Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces bloodfeeding and fecundity. J. Invert. Path, (In press)

SCHOLTE, E-J., NG'HABI, K., KIHONDA, J., TAKKEN, W., PAAIJMANS, K., ABDULLA, S., KILLEEN, G.F. and **KNOLS, B.G.J**. An entomopathogenic fungus for control of adult African malaria vectors. *Science*, in press.

VERA M.T., **C. CÁCERES, V. WORNOAYPORN, A. ISLAM, A.S. ROBINSON,** M.H. DE LA VEGA, J. HENDRICHS, and J.P. CAYOL. Mating incompatibility among populations of the South American fruit fly *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) Ann. Entomol. Soc. Am.

6.2 Travel

Staff Member	Destination	Period	Purpose of Travel
A.S. Robinson	Heraklion, GRE	14–18 Jan	Lecture at a graduate course
	Bratislava, SLR	4 Feb	Technical assistance to IZ-SAS.
	New Haven, USA	14–19 April	Gates Foundation proposal for tsetse
	Bratislava, SLR	28 Apr	Technical assistance to IZ-SAS.
	Gainesville, FL, USA	10–12 May	Lecture and laboratory course at Gainesville Training Course
	Bratislava, SLR	22 July	Technical assistance to IZ-SAS.
	Washington DC, USA	18–23 Sept.	Invited talk at Pew Conference on "Biotech Bugs"
	Heraklion, GRE	3–5 Oct	Lecture at graduate course
	Tapachula, MEX	4–8 Dec	Discuss progress MEX/5/027 with counterparts and develop work plans
H. Bossin	Denver, CO, USA	12–26 June	Participate in Biology of Disease Vectors course.
	Nairobi, KEN & Dar Es Salaam, URT	12–19 July	In Kenya: Participate in WG on Strategic Plan to Bridge Lab. and Field Res. in Disease Vector Control. In Tanzania, Visit mosquito project at IHRDC.
C. Caceres	Tel Aviv, ISR	28–30 March	Review/advise/prepare work plan re medfly work (ISR/5/010)
	Sarasota, FL, USA	11–21 May	Cons. Mtg. on Guidelines for emergence, packing, release of sterile flies and participate in 5th Mtg. of WG on Fruit Flies of the Western Hemisphere.
	Tapachula, MEX; Guatemala City, GUA; Lima, PER; Salvador, BRA	14 Oct–10 Nov	In Tapachula, RCM, Scientific Secretary In Guatemala, finalize automatic spreadsheet for medfly facility design. In Brazil, advise on improvements to mass-rearing protocols and review medfly mass-rearing facility design
A. Parker	Bratislava, SLR	4 Feb	Technical assistance to IZ-SAS.
	Addis Ababa, ETH/Tanga, URT	9–20 Feb	In Ethiopia, Review progress in facility construction, advise on equipment and tsetse mass production; In Tanzania, inspection of fly proofing system and advise on tsetse fly rearing.
	Bratislava, SLR	3 Mar	Technical assistance to IZ-SAS.
	Bratislava, SLR	31 Mar	Technical assistance to IZ-SAS.
	Bratislava, SLR	28 Apr	Technical assistance to IZ-SAS.
	Bratislava, SLR	26 May	Technical assistance to IZ-SAS.
	Bratislava, SLR	18 August	Technical assistance to IZ-SAS.
	Bratislava, SLR	14 Sept	Technical assistance to IZ-SAS.
	Bratislava, SLR	14 Oct	Technical assistance to IZ-SAS.
	Bratislava, SLR	9 Nov	Technical assistance to IZ-SAS.
	Bratislava, SLR	8 Dec	Technical assistance to IZ-SAS.

G. Franz	Tapachula, MEX	17–24 Oct	Review project MEX/5/027
B. Knols	Khartoum, Dongola and Merowe, SUD	9–20 Feb	Discuss status of mosquito colonies and attend meeting in the Northern State. Field missions to Dongola and Merowe .
	Davis, CA, USA	16–18 April	To finalize GATES proposal.
	Nairobi, KEN and Dar Es Salaam, URT	13–19 July	In Kenya, rapporteur forWorking group on strategic plan to bridge lab. and field res. in disease vector control. In Tanzania, visit to VIDI Project in Ifakara, according to work plan.
	Washington DC, USA	18–22 Sept.	Invited talk at Pew Conference on "Biotech Bugs
	Khartoum, Dongola and Merowe, SUD	26 Oct–3 Nov	Attend Coord. Meeting, Field missions to Dongola and Merowe
H. Adun	Bratislava, SLR	18 Aug	Technical assistance to IZ-SAS
R. Boigner	Bratislava, SLR	18 Aug	Technical assistance to IZ-SAS
G. Germershausen	Bratislava, SLR	18 Aug	Technical assistance to IZ-SAS
F. Ibantschitz	Bratislava, SLR	18 Aug	Technical assistance to IZ-SAS
A.H. Mohammed	Bratislava, SLR	18 Aug	Technical assistance to IZ-SAS
M. Taher	Bratislava, SLR	5 Feb	Technical assistance to IZ-SAS.

6.3 Fellows

Name	Country	From	To
GEZAHEGN, Mr. A.	ETH/02026P	2003-07-27 -	2004-01-26
OUATTARA, Mr. S.J.E.	BKF/03042P	2003-10-06 -	2004-04-02
SAMOURA, Mr. O.	BKF/03041P	2003-10-06 -	2004-04-02
SAMOURA, Mr. O.	BKF/03041P	2003-04-03 -	2004-06-02
LUBAZIBWA, Mr. K.B.	URT/03016P	2003-10-12 -	2004-01-11
BRAVO SANZANA, Mr. J.A.	CHI/03004	2004-02-02 -	2004-05-01
NGALANDE, Mr. H.	ZAM/03007R	2004-02-02 -	2004-10-01
MAKUMYAVIRI, Mr. M.P.	ZAI/03009P	2004-04-07 -	2004-06-06
MAKUMYAVIRI, Mr. M.P.	ZAI/03009P	2004-06-07 -	2004-06-18
NOBLE, Ms. M.V.	SAF/04010	2004-06-01 -	2004-08-31
ROSENBERG, Mr. A.	SAF/04011	2004-06-01 -	2004-08-31
ABD-ALLA, Ms. M.	EGY/04017	2004-06-15 -	2004-12-14
DHAOUADI, Mr. F.	TUN/03007	2004-09-01 -	2004-11-30
Scientific visitor			
SHOUSTER, Ms. I.	ISR/04010	2004-11-08 -	2004-11-19

6.4 Tsetse Shipments

***Glossina morsitans morsitans*, (Colony size 7,000)**		
Dr. Maudlin	Univ. Edinburgh, UK	2,700
Dr. Lehane	Univ. Liverpool	11,800
Dr. Turner	Univ. Glasgow, UK	3,750
Dr. Tait	Univ. Glasgow, UK	5,000
Dr. Gibson	Univ. Bristol, UK	12,400
Dr. Terblanche	Stellenbosch Univ.	1,000
Dr. Brun	Swiss Tropical Inst.	12,400
Dr. Aksoy	Yale Univ., USA	14,300
Dr. Malele	Univ Bangor, Wales. UK	13,200
Others		450
		77,000
***Glossina pallidipes*, (Colony size 25,000)**		
Dr. Guerin	Univ. Neuchatel, Switzerland	19,400
Dr. Takac	IZ-SAS	81,434
Dr. Aksoy	Yale Univ., USA	12,800
Dr. Terblanche	Stellenbosch Univ	5,300
Dr. Kiragu	KETRI, Kenya	26,300
Dr. Msangi	TTRI, Tanzania	48,840
		195,174
***Glossina palpalis*, (Colony size 5,000)**		
Dr. Maudlin	Univ. Edinburgh, UK	123,850
Dr. Molyneux	Univ. Salford, UK	13,550
Dr. Aksoy	Yale Univ., USA	13,750
Dr. Omoogun	NITR, Kaduna, Nigeria	13,150
		53,300
***Glossina brevipalpis*, (Colony size 4,000)**		
Dr. Kappmeier	OVI, South Africa	38,985
Dr. Aksoy	Yale Univ., USA	6,700
Dr. Guerin	Univ. Neuchatel, Switzerland	6,750
Dr. Maudlin	Univ. Edinburgh, UK	2,800
		55,235
***Glossina fuscipes*, (Colony size 6,000)**		
Dr. Guerin	Univ. Neuchatel, Switzerland	7,700
Dr. Okedi	LIRI, Uganda	39,290
Dr. Averswald	Univ. Cape Town, South Africa	12,400
Dr. Takac	IZ-SAS	25,990
Dr. Aksoy	Yale Univ., USA	7,500
		92,880
***Glossina morsitans centralis*, (Colony size 5000)**		
Dr. Terblanche	Stellenbosch Univ	700
Dr. Takac	IZ-SAS	27,328
		28,028
	GRAND TOTAL	**501,617**

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